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OZONATION OF PULP MILL WASTEWATER AND MOLECULAR WEIGHT DISTRIBUTION STUDIES

BY

MURRAY HAROLD CARMICHAEL ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE**

IN

ENVIRONMENTAL SCIENCE

DEPARTMENT OF **CIVIL ENGINEERING**

EDMONTON, ALBERTA

FALL 1994

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **OZONATION OF PULP MILL WASTEWATER AND MOLECULAR WEIGHT DISTRIBUTION STUDIES** submitted by **MURRAY HAROLD CARMICHAEL** in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE** in **ENVIRONMENTAL SCIENCE.**

Dr. G.R. Finch

JLeonard

Dr. J. Leonard

James A Plambeck

Dr. J. Plambeck

Dr. T. Davis

Date: 4 Oct 94

DEDICATION

To my Wife **Marcia**

and my Daughter **Sasha**

for their Love and Support.

ABSTRACT

The changes in pulp mill wastewater during secondary treatment and after ozone treatment of the secondary effluent were studied. Molecular weight distribution using high performance liquid chromatography (HPLC) was used as the main analytical tool for these comparisons. Other parameters such as color, BOD_5, COD and TOC were also measured and were used to compare with the changes in the molecular weight distributions of wastewater samples. Ultrafiltration of the secondary effluent and the ozonated secondary effluent was also carried out in order to further study the change in wastewater upon ozonation.

Aqueous size exclusion chromatography was carried out on an organic polymer column using a 25 mM carbonate buffer (pH = 10.3) as the mobile phase. Three types of standards were used for calibrations: (1)Pullulans (polytrisaccharides), (2)polyethylene glycols and (3)peptides. The molecular weight distribution calibration data indicated that the molecular size exclusion results correlated very well to molecular weight exclusion results under the chromatographic conditions used.

The reduction in molecular weight distribution area was much lower than the reduction in total organic carbon from the primary to secondary effluent. This indicated a lack of detection of lower molecular weight compounds with the UV-detector.

Four ultrafiltration fractions from the secondary effluent were obtained using membranes with molecular weight cut-offs of 1000, 5000 and 10000 daltons. The color increased with increasing molecular weight of the secondary effluent ultrafiltration fractions. A straight line relationship was found between the color/TOC ratio and the weighted average molecular weights of the secondary effluent ultrafiltration fractions. Molecular weight distributions of the ultrafiltration fractions of ASB effluent showed a lack of correlation to the manufacturer's cut-off numbers. This was not unexpected as the actual rejection coefficient of the membranes is sample specific.

Ozone doses of 84.8, 111.6 and 192.4 mg O_3/L were carried out on pulp mill secondary effluents using two ozone reactors. A straight line relationship was found between the change in molecular weight distribution areas of ozonated secondary effluents and the ozone dose applied. A comparison of TOC and MWD results indicated that there were lower molecular weight compounds in the ozonated effluent that were not detected by the UV detector. COD results further confirmed this observation. This was due to lower UV absorptivities of chemical bonds oxidized by ozone. The weighted average molecular weight of the effluents decreased upon ozonation. The higher the molecular weight ultrafiltration fraction, the higher the decrease in distribution area upon ozonation. This was due to a higher reactivity towards ozone for larger molecules that have an abundance of unsaturated bonds.

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LIST OF ABBREVIATIONS

Å	Angstrom
Ac	Acetate
admt	air dried metric tonne
adt	air dried tonne
Ag_2SO_4	Silver sulfate
Amm Ac	Ammonium acetate
AOX	Adsorbable Organic Halides
AS	Activated Sludge
ASB	Aerated Stabilization Basin
BCTMP	Bleached-Chemi-Thermo-Mechanical Pulping
BOD_5	5 - day Biochemical Oxygen Demand
°C	degrees Centigrade
ClO_2	Chlorine dioxide
CO_2	Carbon dioxide
COD	Chemical Oxygen Demand
ΔCOD	Change in Chemical Oxygen Demand
CTMP	Chemi-Thermo-Mechanical Pulping
cu	color units
Da	Dalton
DAD	diode array detector
DMF	Dimethyl formamide
DOC	Dissolved Organic Carbon
eq	equivalents
Eff	Effluent
F1	<1,000 MWCO Ultrafiltration fraction
F2	1,000 to 5,000 MWCO Ultrafiltration fraction
F3	5,000 to 10,000 MWCO Ultrafiltration fraction
F4	>10,000 MWCO Ultrafiltration fraction
FAS	Ferric ammonium sulfate
gal	gallons
Gu	Guanidine
H_2O	water
H_2O_2	Hydrogen peroxide

H_2SO_3	Sulfurous acid
H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
HMM	High Molecular Mass
HPLC	High Performance Liquid Chromatography
HPSEC	High Performance Size Exclusion Chromatography
Inf	Influent
$K_2Cr_2O_7$	Pottasium dichromate
kDa	kilodalton
kg	kilogram
KHP	Potassium hydrogen phthalate
KI	Potassium iodide
L	liters
LC_{50}	Lethal concentration for 50% of test fish
LiCl	Lithium chloride
Lin Reg	Linear Regression
M	molar
m	meter
MeOH	Methanol
mg	milligrams
min	minutes
mL	milliliter
mM	millimolar
MS	Mass Spectrometry
MWCO	Molecular Weight Cut Off
MWD	Molecular Weight Distribution
N	Nitrogen
Na_2S	Sodium sulfide
NaCl	Sodium chloride
NAMW	Number Average Molecular Weight
NaOH	Sodium hydroxide
NH_3	Ammonia
nm	nanometers
O_2	Oxygen
O_3	Ozone
ODFW	Ozone Demand Free Water

P	Phosphorus
PEG	Polyethylene glycol
Pept	Peptide
ppm	parts per million
psi	pounds per square inch
Pull	Pullulan
SEC	Size Exclusion Chromatography
std dev	standard deviation
THF	Tetrahydrofuran
TIC	Total Inorganic Carbon
TMP	Thermo-Mechanical Pulping
TOC	Total Organic Carbon
ΔTOC	Change in Total Organic Carbon
tris	tris(hydroxymethyl)aminomethane
TSS	Total Suspended Solids
UF	Ultrafiltration
μL	microliter
μm	micrometer
UV	Ultraviolet
UV-Vis	Ultraviolet-Visible
VPO	Vapor Pressure Osmometry
WAMW	Weighted Average Molecular Weight

1. INTRODUCTION

1.1. Background

Pulp mill wastewater has been a major concern over the years, not only in Canada but in many developed nations throughout the world. One of the biggest problems being the reduction of water pollution from older pulp and paper mills. The environmental impacts associated with pulp mill wastewater include parameters such as suspended solids, biological oxygen demand (BOD_5), chemical oxygen demand (COD), adsorbable organic halides (AOX) and toxicity (acute and chronic). There are also aesthetic impacts associated with this wastewater such as color and odor, which may or may not have environmental impacts.

Suspended solids consist of fine bark particles, silt from the wood room, fiber particles from pulp and paper mill operations and coating and filling materials such as talc, clays, calcium carbonate and titanium oxide (Schmidtke, 1992). The suspended solids can be controlled using primary settling tanks or mechanical clarifiers and are also minimized by long term settling in lagoons. The BOD_5 and COD of pulp mill wastewaters are caused by soluble organics such as wood sugars, carbohydrates, soluble lignin degradation compounds and compounds from municipal waste that may have been added to the wastewater stream. The BOD_5 and COD can be controlled via a secondary treatment step usually involving aerobic methods such as activated sludge tanks or aerated lagoons. Adsorbable organic halides (AOX) are formed from chlorine bleaching of pulp, and are partially associated with acute and chronic toxicity. The AOX can be controlled via chlorine substitution methods during the bleaching process, such as hydrogen peroxide and chlorine dioxide. The acute toxicity associated with pulp mill wastewater and/or added municipal wastewater (bacterial or viral activity) is normally controlled via secondary treatment. Color, COD and odor are controlled using methods such as ozone (O_3) or ozone in combination with microbiological treatment and chemical adsorption, either before or after secondary treatment.

Ozone treatment is one of the methods researched in this project. Previous studies have shown that ozone treatment of pulp mill effluent vastly improves color, odor and foaming characteristics. Ozone treatment has also been shown to improve microbiological treatability of the effluent. Other advantages of ozone treatment are the process is simple and it can be incorporated into existing wastewater treatment systems with minor modifications. Two of the disadvantages of ozone treatment are: (1) high capital and operating costs, and (2) high ozone demands for pulp mill wastewaters. The high operating costs can be minimized via improved efficiency in ozone generation. The high ozone demands can be minimized via improved efficiency in ozone utilization (i.e., better ozone contact systems). As the regulations for pulp mill wastewater become more stringent and the reuse of water becomes imperative, ozone will become an attractive addition to existing effluent treatments.

1.2. Objectives

The objectives of this study were as follows:

1) To establish an HPLC analytical method that gives accurate and reproducible molecular weight distributions with regard to pulp mill wastewaters, and ozonated pulp mill wastewaters. This method was developed from previously established methods in the literature. The method developed could then be used in future pulp mill wastewater studies in order to give a better understanding of treatment methods.

2) To compare primary pulp mill effluent with secondary pulp mill effluent using molecular weight distribution analysis along with TOC, COD, BOD_5 and color. This analysis should give a clear picture of what is happening during the microbiological treatment of the primary effluent.

3) To analyze the ultrafiltration fractions of the secondary effluent for color, BOD_5, COD and TOC, along with molecular weight distributions. The results should establish some correlations between the various analyses.

4) A correlation between the molecular weight distributions of the ultrafiltration fractions of the wastewaters and the molecular weight cut-offs of the ultrafiltration membranes, will also be established. The results would give an understanding of the ultrafiltration performance of such a complex mixture of organics.

5) To compare secondary pulp mill effluent with ozonated secondary pulp mill effluent using molecular weight distribution analysis along with TOC, COD, and color. These results, along with previous literature results, should give further insight into the ozonation of pulp mill wastewaters.

1.3. Scope of Study

The research in this project is a part of a larger project funded by Weyerhaeuser Canada Ltd. This part of that larger project was designed to look at the changes in pulp mill wastewater during secondary treatment and after ozone treatment of the secondary effluent. Molecular weight distribution using high performance liquid chromatography (HPLC) was used as the main analytical tool for these comparisons. Other parameters such as color, BOD_5, COD and TOC were also measured and were used to compare with the changes in the molecular weight distributions of wastewater samples. The research was carried out in the following steps:

1) Color, BOD_5, COD, and TOC characterization of pulp mill primary and secondary effluent.

2) Ultrafiltration of the secondary effluent

3) Color, BOD_5, COD, and TOC characterization of the ultrafiltration fractions of the secondary effluent.

4) Ozonation of the secondary effluent.

5) Ultrafiltration of the ozonated secondary effluent.

6) Color, COD & TOC characterization of the ozonated pulp mill secondary effluent and the ultrafiltration fractions.

7) Development of the molecular weight distribution method.

6) Molecular weight distribution analysis of the wastewaters, ozonated wastewaters and ultrafiltration fractions.

2. LITERATURE REVIEW

2.1. Pulp Mill Operations

2.1.1. Environmental Impacts and Regulations

Concerns over the impacts of pulp mill affluent on the receiving environment have been inherent since the late nineteenth century when the industry first started in Canada. It has been estimated that the pulp and paper industry is responsible for 50% of all the waste dumped into Canadian waters (Canada, 1970). The pulp and paper industry is the cause of 7 of 17 areas of concern identified by the Great Lakes Water Quality Board, in Ontario (Sinclair, 1990).

The effluent discharged from pulp mills into water causes oxygen depletion (BOD_5), disseminates non-filterable residues (suspended solids) and adds known (COD, AOXs, toxic compounds) and unknown compounds that often have adverse effects on aquatic organisms, and if present in sufficient quantities can be lethal (Sinclair, 1990). It has been estimated that one kraft pulp mill discharges BOD_5 compounds at a rate equal to the amount generated from a city's municipal waste with a population greater than 120,000 (Pearse et al., 1985 and Bonsor et al., 1988). Other environmental impacts from pulp mills are associated with odor, fish flesh tainting, acidity, slime growth, thermal effects, foam, scum and color (Environment Canada, 1983). The inherent production and discharge of persistent organic compounds are also potential environmental impacts (Environment Canada, 1987).

As part of an industry that operates worldwide, the Canadian pulp and paper industry has helped develop technologies that have made the mills less threatening to the environment (Sinclair, 1990). Some of this has been done independently by the industry in its own self-interest and that of the public, but the majority has been done in cooperation with government or at the urging of government regulating agencies (Sinclair, 1990). More efficient processing techniques have reduced waste discharges and the mills have monitored and reported their effluent discharges in the general public interest (Sinclair, 1990).

In the early 1960s many felt that although the Fisheries Act could regulate industrial pollution, the Act was limited, even when penalty fines were increased in 1960 (Parlour, 1981). Therefore, during the late sixty's government, the public and the pulp and paper industry were involved in making amendments to the fisheries act, so as to address these concerns. The Fisheries Act amendments received final approval in June 1971. These amendments expanded power to protect fish environment, and set national effluent standards based on technical feasibility especially for the pulp and paper industry effluent (Lindsay, 1993). The permitted deposits of allowable discharges, represented by numerical values in the regulations, were established by a task force comprising federal, provincial and industry representatives. This replaced the original, completely federal task force (Keays and Jackson, 1972). The three levels of compliance included a more stringent standard for new mills, a somewhat less restrictive target for existing (old) mills and a standard somewhere in between the new and old for existing mills that undergo modifications (Sinclair, 1990). The standards were to be enforced on the basis of monitoring information collected at individual mills.

The pulp and paper regulatory package, released in December 1991 by Environment Canada, Fisheries and Oceans, and Health and Welfare Canada proposed changes to the Fisheries Act and the Canadian Environmental Protection Act (CEPPA) (Canada, 1991). The package was developed from extensive consultation with the Canadian Pulp and Paper Association, other federal departments, non-governmental environmental organizations and 14 public meetings across Canada (Canada, 1991). The proposed amendments to regulations under the Fisheries Act included setting new limits on effluent discharges, establishing new monitoring procedures and extending regulations to all mills in Canada (Lindsay, 1993). These proposals yielded amendments to the Fisheries Act Pulp and Paper regulations, which came into effect July 1, 1992.

The Canadian Environmental Protection Act (CEPA) regulations play a role in controlling both toxic chemicals used and by-products emanating from the pulp and paper industry, i.e., dioxins and furan (Lindsay, 1993). Toxic substances (chemicals, effluent and wastes) are defined as being harmful to the environment or constituting a danger to human health. Enacted in June of 1988, CEPA allows for the development of regulations restricting substances deemed toxic under the Act. It consolidated some earlier federal environmental legislation, including the Environmental contaminants Act, the Canada Water, the Clean Air Act, the Ocean Dumping, Control Act, and the Department of Environment Act (Phillips, 1990).

The 1991 Pulp and Paper Regulatory package included new regulations under CEPA. These established new standards for dioxin/furan levels, and an immediate ban on the use of contaminated defoamers on woodchips containing pentachlorophenol was to be regulated. Pulp mills operating prior to June 1, 1990, are required to implement process changes by January 1, 1994, to prevent the formation of dioxins/furan (Lindsay, 1993). Mills built after June 1, 1990, were asked to comply by July 1, 1992. Any measurable amount of dioxins/furan is considered a violation.

In Alberta on December 2, 1988, the Minister of Environment announced new standards for pulp mill developments that put Alberta mills amongst world leaders in controlling the production of dioxins and other organic compounds and in minimizing their release into the environment (Alberta Environment, 1989). Alberta will require expanding or new mills to incorporate all the latest technologies including extended delignification, oxygen delignification and chlorine dioxide substitution in the first chlorine stage of bleaching (Alberta Environment, 1989). The bleaching process creates organic halogens, including dioxins, and the Alberta standards are designed to minimize or eliminate these priority pollutants (Alberta Environment, 1989). Under the Alberta Clean Water Act, Alberta Environment sets specific standards for BOD_5, total suspended solids, color dissolved oxygen, pH, resin acids, acute toxicity and AOXs.

2.1.2. Pulping Processes

Wood is composed of cellulose (fiber), lignin, hemicellulose and extractives. Each of these constituents produces different types of waste as follows (Hall, 1992).

Table 1: Wood Composition and Waste Characteristics

Wood Component	% Composition	Waste Component
Cellulose	45	Fiber (suspended solids)
Lignin	25	Chromophores (color)
Hemicellulose	25	BOD_5
Extractives	5	Toxicity

Pulp is formed from the cellulose fibers. The pulping process used determines the type of waste produced (Schmidtke, 1992). The pulping methods used in the industry are described below:

1. Chemical pulping: This process separates the lignin, hemicellulose and extractives from the wood fibers chemically. The kraft process and the sulfite process are the two major types of chemical pulping. In the kraft process, sodium hydroxide (NaOH) and sodium sulfide (Na_2S) are used to separate the fibers. The sulfite process uses alkaline oxides and sulfurous acid (H_2SO_3) formed from dissolving sulfur dioxide (SO_2). Chemical recovery is an integral part of the kraft process, whereas there is no chemical recovery in the sulfite process (Schmidtke, 1992). Therefore, the BOD_5 is much higher in the sulfite process (Schmidtke, 1992). In both processes suspended solids and color are problems in the effluent while foam and toxicity are larger in the kraft effluent (Schmidtke, 1992).

2. Semi-Chemical Pulping: In this process the wood is cooked for a limited time period, which results in partial delignification. This improves the pulp yield and minimizes degradation of the cellulose. There is no chemical recovery in this process, which yields BOD_5/tonne of pulp that is somewhere between the kraft and sulfite processes (Schmidtke, 1992). Effluent problems are suspended solids and color to a lesser degree than chemical pulping (Schmidtke, 1992).

3. Mechanical Pulping: The pulp fibers are released by mechanically grinding the wood. Most of the lignin and hemicellulose remains with the pulp fibers, which yields a low BOD_5 in the wastewater (Schmidtke, 1992). Major disadvantages of this process compared to the others are a lack of pulp strength, high energy costs

and highly labor intensive (Sinclair, 1990). Suspended solids are a major problem with this process (Schmidtke, 1992).

4. Thermo-Mechanical Pulping (TMP): In this process the wood is steamed under pressure prior to and during the mechanical pulping process. TMP requires less energy than mechanical pulping. TMP effluent has lower suspended solids and higher BOD_5 than mechanical pulping.

5. Chemi-Thermo Mechanical Pulping (CTMP): In this process pulping chemicals are added to wood prior to or during a pre-steaming step. This process uses a lower volume of water than chemical pulping and therefore yields higher BOD_5 and toxicity in the effluent (Hall, 1992). Suspended solids are also a problem.

6. Bleached-Chemi-Thermo-Mechanical Pulping (BCTMP): This process is the same as CTMP except that a bleaching step is added to brighten the pulp. While the color of the effluent in this process is lower than in chemical pulping, the BOD_5 and toxicity are higher. As in CTMP, suspended solids are also a problem in the effluent.

2.1.3. Bleaching Operations

Due to its dark color, chemical pulp requires bleaching before further use. The conventional bleaching process using chlorine produces one half of the BOD_5 and the majority of toxicity and color in the mill effluent (EPS, 1984). Chlorine bleaching of chemical pulp carries approximately 7% of the weight of the pulp discharged to the sewer in the form of a wide variety of compounds, including organochlorines (Bonsor, et al., 1988).

Kraft process pulp mills exclusively used chlorine and chlorine compounds for their bleaching, prior to 1970. Since 1970, oxygen has partially been used for bleaching operations in the form of oxygen delignification. Oxygen delignification is advantageous in reducing organochlorines in BOD_5 in the mill effluent. It is used to reduce the Kappa number (measure of the contents of ligneous and related organic material) of the kraft pulp by about 50%, which results in reduction of about 40% in the quantity of chloro-organics generated in the subsequent bleaching operations (Bonsor et al., 1988). The substances removed from the wood during oxygen delignification are recycled to the chemical recovery system. This permits incineration of organics, which add to BOD_5, organochlorines, color and toxicity in the bleach effluent. The process of oxygen delignification has also shown a reduction in mutagenicity of chlorinated effluent (Germgard et al., 1985).

Bonsor et al. (1988) analyzed the following concerns with oxygen delignification based on the literature and on hand experience available:

- difficult to operate

- high operational control

- oxygen is hazardous
- future chemical costs are unknown.

They concluded that the aforementioned benefits far outweigh these technical concerns, which can be resolved through improved process control techniques.

It is common practice for kraft mills to substitute 5 to 15% of chlorine dioxide (ClO_2) in the first stage of the bleaching plant (Bonsor et al., 1988). The main advantages of chlorine dioxide substitution are the reduction of AOX, toxicants and mutagenicity in the bleach effluent. A substitution of 100% chlorine dioxide in the bleaching operation drops the mutagenicity of the effluent at no higher than in the control tests (Bonsor et al., 1988). In the bleaching substitution 1 kg of chlorine dioxide replaces 2.63 kg of molecular chlorine. Hence, there is a net reduction in the amount of chlorine used and there is a reduction in the amount of chlorinated organics produced.

2.1.4. Pulp Mill Wastewater Treatment Processes

Although in-plant techniques improve the quality of the effluent, external treatment processes must be used in order to achieve regulatory standards. This external treatment usually consists of pretreatment, primary treatment and secondary treatment. Tertiary treatments such as ozone are less common, but are becoming more attractive with increasing research into further color and organics (COD) removals.

In pretreatment, a grit chamber is used to separate the organics from the inorganics (Casey, 1980). The grit chamber is a gravity settling tank that removes inorganic ash, grit, sand, and gravel in order to reduce equipment abrasion and pump damage. Another important component of pretreatment is neutralization of the mill effluent. If not neutralized, the mill effluent pH can cause severe corrosion, upset the secondary treatment (biological) and negatively impact the receiving stream. Neutralization is achieved by using waste chemicals and appropriately mixing selected effluent streams.

2.1.4.1. Primary Treatment

Primary treatment is a physical process that removes approximately 95% settleable solids, 70-90% suspended solids and approximately 10% of the BOD_5 in the mill effluent (Schmidtke, 1992). This is accomplished in a gravity clarifier, consisting of a circular tank equipped with a sludge removal mechanism.

In some mills such as the Slave Lake, BCTMP mill in Alberta, flotation clarifiers are used (Lindsay, 1993). In flotation clarifiers pressurized air is introduced through an upflow unit as the effluent enters the bottom of the tank. Supersaturated air bubbles carry suspended solids to the surface where a scraper separates the particles from the effluent overflowing from the unit. Flotation clarifiers can remove up to 98% total suspended solids (Casey 1980).

Primary sedimentation also has the advantage of removing 40 to 50% of influent phosphorous from a pulp and paper mill (Vuoriranta and Hynninen, 1989).

2.1.4.2. Secondary Treatment

Secondary treatment is a biological process that uses micro-organisms to consume organic wastes in the effluent. The objectives of this process are to reduce the effluent BOD_5 by 70 to 95% and to reduce the effluent toxicity. These biological systems tend to be more effective in removing low molecular weight organics (i.e., methanol) than high molecular weight organics (i.e., resin acids and chlorinated lignins). The three most common secondary treatment processes are natural stabilization lagoons, aerated stabilization lagoons and activated sludge. Less common processes are anaerobic treatment and trickling filters.

Natural stabilization lagoons, also called long retention time lagoons, were created in the southern United States. In these areas land was inexpensive and effluent discharge into streams had to be limited during low summer flows. The least expensive and most common system of natural stabilization lagoons is a series of holding ponds. These ponds have to be large enough to retain the effluent until natural decomposition processes breakdown the organic wastes. BOD_5 can be reduced 50 to 90% with a retention time of 20 to 60 days in these lagoons and a loading of approximately 5×10^{-5} $kg/m^2/day$ (Casey, 1980). Although operating costs are very low for long retention time lagoons they require extensive acreage, which translates to high capital costs where land prices are high. Lower temperatures in Canada drop the BOD_5 reduction in these lagoons to less than 50% (Schmidtke, 1992). Therefore, long retention time lagoons have problems meeting regulatory standards. Due to these problems long retention time lagoons have generally been replaced with Aerated Stabilization Basin in the pulp industry.

Aerated Stabilization Basins (ASB) incorporate mechanical aeration in lagoons with a retention time of six to ten days (Schmidtke, 1992). The first surface aerated system in the pulp and paper industry was developed by the Reigel paper company in a North Carolina kraft mill in 1964, and subsequently won a State Wildlife Federation Award (Council on Economic Priorities, 1972). This type of system has become common in western Canada (McAllen, 1989). ASBs have BOD_5 removal efficiencies of 80 to 95% and tend to have better toxicity removal than other systems. Total resins can be reduced to as low as 1.30 mg/L in the ASB effluent in cold climates (McAllen, 1989). Nutrients (BOD_5:N:P = 100:5:1) are normally added to ASBs in the form of urea and phosphoric acid in order to increase the biodegradation efficiency. One disadvantage of ASBs is decreasing BOD_5 removal with decreasing temperatures. A 10 °C temperature drop requires a 35% detention time increase (Casey, 1980). Staging the ASB to keep high temperatures in the first stage, minimizes temperature effects.

Activated sludge (AS) technology was borrowed from municipal waste treatment methods. AS systems can be designed with air or pure oxygen (McAllen, 1989). If space is a major concern or land costs are high AS systems are an attractive alternative to ASBs (Schmidtke, 1992). Biological solids are recycled in the AS system in order to achieve a

90 to 95% BOD_5 removal (McAllen, 1989). Substantial toxicity and COD removals are also achieved (McAllen, 1989). BOD_5 and AOX removals with both types of activated sludge systems are generally higher than ASBs (McAllen, 1989). AS systems are less susceptible to winter operating conditions, due to short retention times of 4 to 6 hours (Schmidtke, 1992). Two major disadvantages of AS systems are the disposal of a large amount of biological solids and high operational costs (Schmidtke 1992). Due to these problems and some operational problems, (i.e., bulking) AS systems have been slow to be incorporated into the pulp industry.

Anaerobic lagoons and trickling filters are not widely used in the pulp and paper industry (Schmidtke, 1992). Both systems have problems achieving regulatory standards for BOD_5 and toxicity.

2.1.4.3. Ozone Treatment Studies

Ozone treatment of pulp mill effluent has been shown to be effective in removing color, odor and forming characteristics along with improving the biological treatability of the effluent. Ozone (O_3) has the second highest chemical oxidation potential known. Only fluorine (F_2) has a higher oxidation potential but it has no pollution control applications. Ozone, therefore, has the power to chemically attack and alter or destroy organic pollutants at much faster rates and lower dosages than other common oxidants (Nebel et al., 1974).

Bauman and Lutz (1974) used a 5 gal/min pilot plant to study the ozonation of a kraft mill effluent. The effluent was collected from an integrated kraft mill, producing about 500 tons/day of fully bleached pulp and about 600 tons/day of fine papers. During 96 hours of continuous running, (20 ppm ozone for 48 hours and 30 ppm ozone for 48 hours) color, BOD_5, COD and suspended solids were analyzed on 8 hour composite samples of the secondary effluent feed and the ozonated effluent. The following average changes were observed during this run: BOD_5 increased 105%; COD was reduced 12%; and suspended solids were reduced by 22%. In other runs 60-70% reduction in the color of the secondary effluent was achieved with application of 30 to 40 ppm ozone.

Bauman and Lutz (1974) developed a regression equation using multiple regression analysis of the effluent parameters as follows:

$$\% \text{ Color Removed} = 67.4 + 0.0552 \text{ (ppm } O_3) - 0.111 \text{ (COD)}$$

$$- 0.0686 \text{ (initial color)} + 0.159 \text{ (suspended solids)}$$

$$+ 0.144 \text{ (units of color lost).}$$

Further studies were also done with extended ozonation 80% color reduction was achieved at 80 ppm ozone and even after nearly 300 ppm ozone there was 30 ppm residual color. After going from 10 to 24 ppm at 40 ppm ozone, the BOD_5 level then remained between 20 and 25 ppm at up to 200 ppm ozone, and subsequently dropped to 10 ppm at 280 ppm ozone. They concluded that the rise in BOD_5 was probably the result of ozone splitting large non-biodegradable organic molecules into smaller fragments that are biodegradable. They also saw good disinfection results as ~ 99% of the coliform bacteria were killed by 40 ppm ozone and 99% of the total bacteria were killed by 100 ppm ozone.

Nebel et al. (1974) studied the effect of ozonation on the secondary effluents from four different mills. Mill A and mill B are kraft pulp and paper mills producing fine papers. At a 70 ppm ozone dose mill A effluent had a 81% color removal, 37% COD removal, 98% total bacteria removal and a 93% coliform bacteria removal. At an 81 ppm ozone dose mill B effluent had a 78% color removal, 29% COD removal and a 50% decrease in turbidity.

Mill C is a bleach board mill using both kraft and neutral sulfite pulp processes. With this effluent 143 ppm ozone achieved a 88% color removal, 21% COD removal, 67% turbidity removal, 16% BOD_5 removal and a 99% total bacteria decrease. The effect of various ozone doses on the BOD_5 was also studied using mill C effluent. As the ozone dose increased, the initial BOD_5 content decreased rapidly, but it increased at high ozone dosage levels, and finally reduced again at still higher ozone doses. The BOD_5 level never increased higher than the initial values as in the Bauman and Lutz (1974) study. Nebel et al. (1974) concluded that the initial BOD_5 reduction is the result of ozone oxidizing the readily biodegradable compounds in the effluent and the subsequent BOD_5 increase is due to the ozone attacking refractory compounds such as degraded lignins making them biodegradable. They interpreted that this phenomenon is achieved because ozone introduces oxygen-containing functional groups on the refractory materials, which afford bacteria a site where metabolism can commence resulting in the increase BOD_5. Then the continued application of ozone further oxidizes these materials, yielding the observed decrease in BOD_5.

Melnyk and Netzer (1975) reported a kinetic study of the reactions between ozone and those lignin compounds that produce an intense color in wastewaters originating from kraft pulping and pulp bleaching processes. The experiments were conducted in a batch reactor and the kinetic model was developed for color removal with the following steps:

1. Color is assumed to be contributed by two species, identified as "A" and "B", both having different reactivities with ozone

2. Each species is assumed to react with soluble ozone as a first order rate process

   $$C_A = C_{Ao} e^{-k_A t} \quad \text{and} \quad C_B = C_{Bo} e^{-k_B t}$$

   with t = time and subscript "o" denoting initial concentrations

3. fo is the fraction of total color contributed by species A,

   $$C_{Ao} = f_o C_{To} \quad \text{and} \quad C_{Bo} = (1-f_o) C_{To}$$

4. Substituting the fraction equations into the rate equations an equation for the total color results

$$C_T = f_o C_{To}e^{-k_A t} + (1\text{-}f_o) C_{To}e^{-k_B t}$$

5. Assuming that the color removal rates for both species are linearly dependent upon the soluble ozone concentration

$$k_A = k_A' C_{O_3} \quad \text{and} \quad k_B = k_B' C_{O_3}$$

6. The final form of the model becomes:

$$C_T = f_o C_{To}e^{-(k_A' C_{O_3} t)} + (1 - f_o) C_{To}e^{-(k_B' C_{O_3} t)}$$

The two species in the model were not identified and, in fact, could be different chemical bonds in the same molecule. The results based on the model showed the more reactive species was responsible for 65 to 87% of the total color. Melnyk and Netzer (1975) also observed that the percentage decrease in COD in somewhat less than the corresponding decrease in color intensity for the same sample. They concluded that the color removal was not accomplished by complete oxidation of the chromogenic lignins to carbon dioxide and water, but rather by a change in their molecular structure.

Furgason et al. (1974) studied ozonation of a kraft mill effluent with both lab and pilot scale units. The pilot scale results indicated that complete noxious odor elimination, color reduction of 75-80% and a COD reduction of 10-15% can be achieved when 200 mg/L of ozone is utilized. The residence time of ozone contact was 10 - 12 minutes.

Smith and Furgason (1975) studied the effect of ozonation on the ability of microorganisms to biodegrade kraft pulp mill liquid waste. Oxygen uptake rates on ozonated and unozonated waste were looked at. Also, one series of experiments was run on diluted wastes to evaluate the effect of toxicity. The results of the various experiments indicated that ozonated kraft mill waste material is more readily consumed by bacteria than untreated waste. They deduced that this increase was due to a reduction in toxicity of the material rather than a significant alteration in the chemical structure from ozone oxidation. They stated that for the ozone to actually sever the lignin molecules and break them into smaller, more biodegradable materials a prolonged ozone treatment would be required which is probably out of the range of economic feasibility.

In another study, Ng et al. (1978) looked at the removal of BOD_5 and color during the ozone treatment of kraft mill effluents. They showed that the removal of color and BOD_5 could be predicted by the following mathematical relations:

$$\ln \text{(color removed)} = 0.72 \ln \text{(ozone applied)} + 3.78$$

$$\ln (BOD_5 \text{ removed}) = 0.86 \ln \text{(ozone applied)} + 0.26$$

Applications of 48 mg/L ozone removed about 28 to 34% of the color while a 90% color reduction was obtained with an ozone dose between 150 and 175 mg/L. BOD_5 increased by 5 to 6% upon initial treatment with 4 mg/L ozone, it then decreased by 20 to 22% with an ozone dose of 48 mg/L, and decreased to 61 to 66% with an ozone dose 150 to 175 mg/L. They deduced that the initial increase of BOD_5 by low level ozone treatment is probably due to degradation of larger molecules to biodegradable molecules which upon further treatment are completely oxidized to carbon dioxide and water.

Ng and et al. (1978) also found that 48 mg/L ozone removed 5 to 6% of TOC and that ozone did not remove toxicity. They also found that pH has a minimal effect on ozone treatment as similar amounts of color and BOD_5 were removed at various pH values ranging from 4.2 to 9.

Prat et al. (1989) researched the ozonation of a bleach stream from a kraft mill for color removal. Their results indicated that there were at least three classes of compounds with different reactivities towards ozone, with regard to color. The first group constituted ~ 90% of the total color and decolorized quickly with ozone, the second group constituted ~ 10% of the total color and decolorized slowly, and the third group of compounds had no color and represented the ozone demand after total decolorization. They estimated that the rate constant for the first group of color causing compounds was 26 times higher than the second group of compounds.

The findings of Prat et al. (1989) correlate quite well with Melnyk and Netzer (1975) in that both deduced that there are two types of compounds or bonds responsible for pulp mill color and that one type reacts much faster with ozone.

Sozanska and Sozanski (1990) conducted experiments in a batch reactor on samples of secondary effluent from pulping processes and paper making. Samples were treated in three ways: ozonation alone, ozonation after alum or lime coagulation, and ozonation after chlorination. Application of the maximum dose of 250 mg/L ozone gave 88 to 90% color removal and 21 to 37% COD removal. They found that after averaging characteristics of experimental series the most effective ozone dose amounted to 80 mg/L while the highest efficiency of color removal achieved with this dose was 0.73% $(1 \text{ mg } O_3/L)^{-1}$. Averaging characteristics revealed that doses ranging between 40 and 150 mg/L ozone yielded identical COD removal efficiencies amounting to 0.20% $(1 \text{ mg } 0_3/L)^{-1}$. For initial values of less than 23 mg/L the BOD_5 increased with ozonation, but for higher initial values the BOD_5 decreased with ozonation.

Sozanska and Sozanski (1990) found that coagulation pretreatment improves the efficiency of color and COD removal. They also found that chlorination pretreatment lowers the efficiency of the ozonation process.

Saugier and Vegega (1991) treated pulp bleach process effluents with ozone to obtain 90% removal of AOXs. 600 ppm ozone was required for 90% removal of color. However, an addition of 100 ppm hydrogen peroxide (H_2O_2) during the ozone treatment improved the color removal considerably, with only a 300 ppm ozone dosage needed for 90% removal.

Hulsey et al. (1991) researched the oxidation of color and organics in pulp and paper mill wastewater by ozone and other oxidation processes such as ozone/UV and ozone/H_2O_2.

The wastewater came from four sources: alkaline bleaching, acidic bleaching, thermomechanical pulping and secondary effluent. Bench tests with the secondary effluent showed that with an ozone dose of 222 mg/L the color removal was 76%. The use of 100 mg/L H_2O_2 along with only 100 mg/L ozone gave a 65% color removal. A bench test on the acid bleaching stream showed 43% color removal after 90 mg/L ozone, 54% color removal after 190 mg/L ozone and 57% color removal after 480 mg/L ozone applied. The TOC was reduced approximately 10% with the 480 mg/L ozone dose.

Roy-Arcand et al. (1991) studied ozone and fungal treatments of a kraft bleaching effluent and also looked at the two treatments combined. Alkaline extraction stage effluent was dialyzed using pore sizes of 1 kilodalton (kDa) and 12-14 kDa and the resulting fractions were also treated. They found that with an ozone treatment of 300 mg/L the complete effluent had a 63% reduction in color. At this same ozone dose the >12-14 kDa fraction had a color removal of 60% and a COD removal of 26%, and the <12-14 kDa fraction had a 78% reduction in color. Their data indicated that the efficiency of ozone in lowering COD is poor in that 950 mg/L ozone only decreased the COD by 14%. They concluded from this that the substrate incorporates only one oxygen atom per ozone molecule. They further deduced that in this alkaline solution the ozone reacts almost exclusively by electrophilic substitutions, resulting in the loss of O_2, rather than by 1, 3 - dipolar cyclo-addition.

Roy-Arcand et al. (1991) found that changing the pH of the solution from 4.5 to 11 did not significantly change color and COD removals during ozonation. Using an ozone dose of 250 mg/L they reported more than 90% removal of various chlorinated phenols and guaiacols from the whole bleach effluent. They also found that combined ozone and fungal (T. versicolor) treatments can be synergistic. A low dose of ozone followed by fungal treatment yielded more rapid decolorization and TOC removal than either treatment produced on its own.

Mohammed and Smith (1991) researched the ozone treatment of effluent from a kraft process pulp mill in a batch reactor using ozone doses from 50 mg/L to 200 mg/L. The batch reactor used consisted of a cylindrical section for holding the effluent and a top spherical section for the ozone/oxygen mixture. The variation in the applied ozone dose was less than 5 mg/L. The bleach and primary effluents were treated with 50 and 100 mg/L ozone doses, in duplicate. The secondary effluent was treated with 50, 100, 150, and 200 mg/L ozone doses, with 6, 6, 2, and 1 replicate experiments for each dose, respectively.

The results from the Mohammed and Smith (1991) paper indicated that 50 and 100 mg O_3/L effectively removed color from the bleach and primary effluents, but did not significantly change the BOD_5. However, with the secondary effluent the BOD_5 was increased by 65% for 50 mg/L ozone doses and 100% for 100 mg/L ozone doses. The corresponding reduction in color for the secondary effluent with these doses was 62% and 82%. They could not establish a clear relationship for the reductions in suspended solids, COD and TOC for various ozone doses.

2.2. Molecular Weight Distribution Research

The standard method of obtaining molecular weight distribution (MWD) of organic compounds is size exclusion chromatography (SEC). This technique is a major liquid chromatography model with the separation based upon the molecular size of the solute in solution (Mant and Hodges, 1991). It is also known as gel-permeation, gel-filtration, steric-exclusion, or gel chromatography. The SEC packing is a gel with an inert originally attained using soft gels such as Sephadex, a crosslinked dextran gel that cannot withstand pressures exceeding 1 or 2 atmospheres. In 1980, semi-rigid (crosslinked hydrophilic polymers gel) and rigid packings (silica gel) were commercialized, which made SEC available in high performance liquid chromatography (Dubin, 1988). SEC via HPLC systems offered over 5,000 theoretical plates per meter and meant better separations at pressures of 2000 kPa or more (Dubin, 1988).

Another method of obtaining MWD data is using ultrafiltration. The molecular weight ranges for each fraction of the sample are determined by the membrane pore sizes in the ultrafiltration unit.

2.2.1. Molecular Weight Distribution of Lignins

Kirk et al. (1969) studied the fractionization of lignin using preparative gel-permeation chromatography. A non-derivitized lignin prepared by enzymatic liberation from sweetgum sapwood was used. Three fractions were obtained using an agarose gel (Bio-Gel A) preparative column and dioxane-water (1:1) mobile phase. The lowest molecular weight fraction was then further fractionated into two molecular weight fractions using the same mobile phase and a column containing crosslinked alkylated dextran gel (Sephadex LH-20). Average molecular weights were then obtained for each of the four fractions using vapor pressure osmometry and membrane osmometry methods. Dimethylformamide (DMF) was used as the solvent for these molecular weight determinations. The weighted average molecular weights for the four fractions were 5000, 2520, 1100 and 400. The unfractionated sample had an average molecular weight of 1250 Da. They also discovered that the intrinsic viscosity of the 2520, 1100 and 400 fractions indicated that these molecules were spherical and that the 5000 fraction had largely asymmetrical molecules. They deduced that the asymmetry of the molecules in the high molecular weight fraction could be due to association effects (i.e., dimerization). The spherical nature of the lignin molecules is very important in SEC work because the exclusion is based on the size of the molecules.

Forss et al. (1976) studied the molecular weight distributions of lignosulfonates, as standards. Lignosulfonates, formed from sulfite pulping, were eluted from a preparative sephadex gel column G-75 using 0.25 M $CaCl_2$ water as the mobile phase. Four lignosulfonate fractions were collected and their average molecular weights were determined using the light scattering method. A straight line calibration plot was then obtained by plotting the logarithm of the average molecular weight vs. the relative retention volume.

Forss et al. (1976) used this method to show the influence of wood species and cooking method on the MWD of spent sulfite liquors. Their data indicated that acid bisulfite cooking of spruce and birch yielded 51% and 15.5% of the MWD higher than 5,000 Da, respectively. Sodium bisulfite cooking of spruce wood gave 42% of the MWD higher than 5,000 Da. Another application of this method was to study the MWD changes associated with the cooking time of pine wood using the kraft process. Their data showed that the mean molecular weight of the kraft lignin samples was low at the early stage of cooking, it then increased as the large lignin fragments dissolved and finally decreased as those fragments depolymerized with increased cooking time. The lignosulfonates and kraft lignins were detected using a spectrophotometer set at a wavelength of 280 mm. Sephadex gel - G-50 was used for the MWD analysis studies.

Conners et al. (1980) looked at association complexes of lignins using SEC. A kraft lignin sample was separated on an Octylsepharose GL-4B open column using dimethylformamide (DMF) as the eluent. A bimodal MWD resulted for the sample under these conditions. When LiCl was added to the DMF at a concentration of 0.1 M, the bimodal elution pattern was replaced by a single broad peak at approximately the same molecular weight range as the second peak eluted with only DMF. Polystyrenes were used for molecular weight markers. These results indicated that the lignin formed association complexes in the pure DMF that were not formed when LiCl was added. The peak of the MWD using LiCl/DMF was at ~2,000 Da. The high and low molecular weight areas of the bimodal MWD of synthetic lignin were collected using DMF. These two fractions were then rechromatographed using 0.1 M LiCl/DMF and they were not of such widely different molecular weight as shown in the MWD with pure DMF. The low molecular weight area from the bimodal MWD does not contain the high MW material, but the high MW fraction does contain low MW material. They concluded, therefore, that the associative effect in DMF does not seem to be solely on the basis of molecular weight. Running the kraft lignin sample on µ-Styragel HPLC columns using dimethyl sulfoxide also indicated a discrete mode of association. Lignin samples were monitored at 280 mm and polystyrenes at 272 mm.

Faix et al. (1981) used SEC to compose the isolation of wood lignins using two types of milling. Milled wood lignins from two different trees of the species Shorea polyspersma were isolated by milling in toluene suspension and by milling in the dry state. Both lignin samples were then acetylated using acetic anhydride/pyridine (1:1 v/v). Both acetylated lignin samples were then run through four microgel HPLC columns (pore sizes 50, 100, 500 and 1000 Å) using tetrahydrofuran (THF) as the mobile phase. The detection was made by a UV-detector at a wavelength of 254 nm and polystyrene standards were used for the MWD calibrations. The lignin isolated in the dry state had a total MWD area approximately 5 Times higher than the lignin isolated by milling in toluene suspension. However, although the yields for the two methods of milling were vastly different the MWDs of each sample had very similar characteristics. The weighted average molecular weights were 5,600 and 6,000 Da for the dry milled lignin and the toluene milled lignin, respectively, and the dispersivities were 2.5 and 2.4. The dispersivity is the ratio of the weighted average molecular weight over the number average molecular weight.

In another study Faix et al. (1980) used the same SEC method described above to compare the MWDs of lignins from various wood species. Milled wood lignins were prepared from the composite wood of spruce, beech, aspen, birch, dabema, bamboo, and roton species.

Pellinen and Salkinoja-Salonen (1985b) used high performance SEC to study the effect of possible adsorption or intermolecular association on the chromatography of lignins and derivitized lignins. Acetylated, silylated and original lignin were run on 2 ultrastyragel columns in series (1000 Å and 500 Å styrene divinylbenzene copolymer) using THF as a mobile phase. Polystyrene standards, model lignin compounds were used to calibrate the molecular weight. The results obtained showed that adsorption or association effects didn't cause any major interferences in the SEC system. The derivitization of the lignins increased their average molecular weights more than was calculated stoichiometrically. They deduced that the derivitization increased the hydrodynamic volume of the lignin molecules or there might be association effects with the derivitized lignin model compounds would be more suitable in the analysis of lignin derivatives than polystyrenes.

Sagfors and Starck (1988) used SEC to separate high molar mass (>1000 Da) lignins from bleached kraft pulp mill effluents. The high molar mass (HMM) lignins were studied using a Sephadex G-50 analytical column with 0.5 M sodium hydroxide as the eluent. The absorbance at a wavelength of 280 nm was continuously monitored by means of a flow-through UV detector. Proteins and polypeptides of known molar mass were used for the MWD calibration.

Their results showed that ~20% of the acid stage effluent and 65-75% of the alkaline stage effluent had a molar mass greater than 1000 Da. They also separated the HMM material using a preparative Sephadex G-25 column with water as the eluent. Upon determination of the absorptivity at 280 nm they were able to show that lignin is the precursor of the HMM components in the alkaline stage and that lignin and carbohydrates are the precursors in the acid stage.

2.2.2. Molecular Weight Distribution of Pulp Mill Wastewater Streams

Ganczarczyk and Obiaga (1974) used SEC to study the mechanism of lignin removal in activated sludge treatment of pulp mill effluents. Sulfite and kraft pulp mill effluents were looked at. They found that in activated sludge treatment of the sulfite pulp mill effluent the lignin was removed as lower molecular fractions of lignosulfonates, and a synthesis of high molecular weight material seemed possible. In treatment of the kraft mill effluents, lignin was removed, in a uniform extent, throughout the whole spectrum of the MWD.

Pellinen and Salkinoja-Salonen (1985a) used aqueous SEC to study the MWDs of bleach effluents. An organic semi-rigid high performance gel column (TSK PW) was used and the performance of several mobile phases was studied. Calibrations and resolution calculations were done with each mobile phase using sodium polystyrenesulfonates as standards. The best combination of calibration linearity and resolution or separation ability was attained with 25 mM sodium bicarbonate buffer with 0.5 g/L of polyethylene glycol (MW = 6000) added. The buffer had a pH of 10.5 and ionic strength of 0.04. Polyethylene glycol was added to minimize hydrogen bonding or hydrophobic adsorption between the column and the bleaching effluent molecules. Using this system they concluded that the MWD corresponded quite well with the MWD obtained using a Bio-Gel P-60 open column with a module phase of 24 mM tris-HCl + 0.1 M NaCl. However, the MWD of the bleaching effluent attained on a Sephadex LH-60 open column with 1% (v/v) acetic acid in DMF differed markedly from the MWD obtained using the aforementioned system. Ultrafiltration fractions of the bleaching liquor were also obtained using a 5000 Da membrane. The weight average molecular weight of the >5,000 Da and <5,000 Da fractions were 7,000 Da and 2,400 Da, respectively.

Jokela and Salkinoja-Salonen (1992) researched the MWDs of organo-halogens in bleached kraft pulp mill effluents. They used nonaqueous SEC on 4 ultrastryragel styrene-divinylbenzene copolymer columns connected in series. The mobile phase used was THF and light absorption was recorded at broad (225-445 nm) and narrow (275-285 nm) bandwidths with a reference wavelength of 450-550 nm. Polystyrene standards and lignin model compounds were used for molecular weight calibrations. They found that over 85% of the chlorinated organics in bleached kraft pulp mill effluents had molecular weights less than 1,000 Da and that the weighted average MW ranged from 300 to 550 Da. Ultrafiltration was also done on the effluent using 1,000 and 10,000 molecular weight cut offs. The peaks of the <1000, 1000-10000, and >10000 fractions using SEC were located at 350, 800 and 2500, respectively which were lower than predicted. In this study aqueous SEC was also attempted using the method described previously (Pellinen and Salkinoja-Salonen, 1985b). They found, however, that the recovery of some chlorophenols was extremely poor with the TSK-PW columns, indicating that there was strong adsorption to the column matrix.

Higashi et al. (1992) studied the SEC of HMM material from bleached kraft mill effluent. The HMM material was extracted from the effluent using methylene chloride and acetonitrile and isolated using ultrafiltration with a 10,000 Da membrane (retentate). High performance SEC was achieved on a Shodex OH-Pak KB-805 (semi rigid organic polymer) column with 10 mM tris (pH = 8) buffer as the mobile phase. Molecular weight calibrations were carried out using protein standards and polysaccharide standards (pullulan P-82). The eluted sample was detected with a UV-detector at a wavelength of 208 nm. The SEC-HPLC of the HMM indicated a molecular mass range from 30 to 300 kDa.

Jokela et al. (1993) researched the effect of biological treatment on halogenated organics in bleached kraft pulp mill effluents using SEC. The SEC method used is described in another study in this report (Jokela and Salkinoja-Salonen, 1992). The organohalogen compounds of wastewaters from 5 pulp mills with different processes exhibited similar MWDs, ranging from 100 to 4000 Da. They found that an anaerobic/aerobic lagoon system removed 58-66% of the organochlorine compounds in the MWD and the full scale activated sludge plants removed 19-55%. Both treatments were slower to remove all size classes of organochlorine molecules while slightly shifting the MWD towards larger molecular weights.

2.2.3. Molecular Weight Distribution of Ozonated Pulp Mill Wastewater

Stern and Gasner (1974) used molecular weight distribution analysis as one of the methods to study the ozonation of lignin. The source of lignin was commercially available acid free pine lignin knows as Indulin AT. Changes in the molecular weight distribution of lignin during ozonation were measured by gel permeation chromatography on a Waters analytical liquid chromatograph with a differential refractometer detector using Corning glass beads with a fractionating range of 300 to 1,200,000 molecular weight. Na_2SO_4 in water was used as the mobile phase and the calibration was carried out using standard dextran. The molecular weight distribution of the starting material had a broad molecular weight peak that ranged from 20,000 Da to 70,000 Da. After the application of 30 milligrams of ozone per gram of lignin about 15% of the reaction mixture had a molecular weight of 1000 Da or less. The molecular weight distribution of this ozonated lignin was also bimodal. In looking at these results they concluded that the bonds most reactive to ozone are located at branch points of the structure, possibly at sites adjacent to the para carbon of the benzene ring that contains other bonds linking subunits together.

Stern and Gasner (1974) then applied 1.5 g O_3/g lignin to the lignin. After this excessive ozone application the molecular weight distribution showed that more than 50% of the organics were completely oxidized to carbon dioxide and water. The distribution was, again, bimodal and it showed that 85% of the remaining organic material was below a molecular weight of 10,000 Da. From these results they concluded that oxalic acid, which was formed earlier, probably oxidizes completely to carbon dioxide and water and that any methanol formed is volatilized. They also concluded that 15% of the high molecular weight lignin remained due to the saturated bonds between phenylpropane subunits being resistant to ozone oxidation.

Mansson and Oster (1988) studied the ozonation of kraft lignin using SEC as one of the analytical techniques. Five ultrastyragel columns were used in series with THF as the eluent and UV detection at 280 nm. The lignin and ozonated lignin samples were methylated before the SEC experiments. 1 gram of lignin was dissolved in 200 mL of distilled water for the ozone runs. The lignin sample was ozonated for 30 minute and 90 minutes using oxygen with an ozone concentration of 1.6 mM O_3/L at a flow rate of 0.5 L/min. The shape of the MWD after 30 minutes of ozonation was very similar to the original lignin MWD, but the quantity of material seen by the detector was slightly less. The weighted average MW was very similar for the original and 30 minutes ozonated lignin samples. However, after 90 minutes of ozonation the MWD was quite a bit narrower than the original and the weighted average molecular weight was lower. The total area of the MWD for the 90 minutes ozonated lignin was also substantially lower. No numbers for these observations were given in this paper so the changes had to be interpreted by visual inspection of the MWDs.

Roy-Arcand et al. (1991) compared ozone and fungal treatments of a kraft bleaching effluent using SEC and other analytical technique. A superose 12 sieve column was used with 0.02 M NaOH + 0.1 M NaCl as the eluent. UV detection was undertaken at a wavelength of 280 nm. After an ozone dose of 250 mg/L the peak area of the MWD was reduced by 37%, but the MWD did not shift and was virtually the same shape.

2.3. Research Needs

The literature search and review for this project showed that there was extensive research undertaken in the ozonation of pulp mill wastewaters. The majority of these projects focused on the changes in standard wastewater parameters such as BOD_5, color and COD. There was also a fair amount of research undertaken in the area of size exclusion chromatography (SEC) analysis of lignin and pulp mill wastewaters.

Some of the areas that are lacking in the literature, according to this review, are as follows:

- Insufficient research was found on the molecular weight distribution (MWD) differences obtained upon ozonation of pulp mill wastewaters.
- Insufficient research was found on comparing the changes in MWDs to the changes in established wastewater parameters such as BOD_5, color, COD and TOC during the biological treatment of pulp mill wastewater.
- No research was found on comparing the changes in MWDs to the changes in established wastewater parameters such as BOD_5, color, COD and TOC during pulp mill wastewater ozone treatment.
- No research was found on the use of ultrafiltration combined with SEC to study the changes in pulp mill wastewater during ozone treatment.

The lack of research in these areas helped to form the objectives for this study. It was hoped that the research undertaken would further contribute to the understanding of pulp mill wastewater biological and ozone treatment, at the molecular level.

3. EXPERIMENTAL PROTOCOL

3.1. Mill Operations

The Weyerhaeuser pulp mill is located near Grande Prairie on the bank of the Wapiti River approximately 30 kilometers from its union with the Smoky River (Lindsay, 1993). The mill was constructed during 1971/72 and the first pulp shipment was in August of 1973. The mill was sold from Proctor and Gamble Cellulose Ltd., to Weyerhaeuser Canada Ltd., in December of 1992.

As required in the License to Operate, monitoring has been conducted to ensure proper operation of the effluent treatment system, which includes primary and secondary treatment (Lindsay, 1993). Monitoring obligations require effluent discharged to the river to continuously record pH, temperature, conductivity, dissolved oxygen and flow rate. Daily or 24-hour composite sampling is required for TSS, BOD_5, pH, COD, conductivity and color. Composite samples (24-hour) are required for NH_3-N and total solids on a weekly basis. The mill is required to measure dissolved oxygen once per week if the river flow drops below 17 m^3/sec and once per day if the flow is below 7 m^3/sec (Lindsay, 1993).

As a result of switching to 100% chlorine dioxide substitution in the bleaching process, the mill reported a drop, in AOX monthly average values from 2.1 kg/adt in 1990 to 0.6 kg/adt in 1992 (Lindsay, 1993). In 1991 the Alberta standard for AOX was 3.0 kg/adt and in 1992 it was 2.5 kg/adt (Lindsay, 1993).

Table 1 on the following page shows the performance data and license limits from 1982-1992 for the Weyerhaeuser pulp mill (Lindsay, 1993). The effluent toxicity testing failure is defined as a 96 hour LC_{50} of less than 100% effluent concentration.

Figure 1 displays a flow diagram of the Weyerhaeuser pulp mill pulping, bleaching and wastewater treatment processes.

Table 2: Weyerhaeuser Canada Ltd., Grande Prairie Pulp Mill plant performance data and license limits from 1982-92 (Lindsay, 1993)

Year	**Production** (Admt/d)*	**Effluent Flow** ('000 m^3/d)	**BOD_5** Mill Effluent (kg/Admt)	***BOD_5*** *Federal Standard (kg/Admt)*	***BOD_5*** *Alberta Standard (kg/Admt)*	**TSS** Mill Effluent (kg/Admt)	***TSS*** *Federal Standard (kg/Admt)*	***TSS*** *Alberta Standard (kg/Admt)*	**Toxicity** Mill Effluent (Pass/total)
1982	778	54.0	4.6	31.2	11.3	7.5	8.0	13.9	1/1
1983	809	53.4	3.2	31.2	11.3	5.4	8.0	13.9	5/5
1984	851	56.6	3.3	31.2	8.7	5.8	8.0	10.7	4/5
1985	851	55.4	4.2	31.2	8.7	7.2	8.0	10.7	4/4
1986	794	58.3	3.6	31.2	8.7	6.4	8.0	10.7	4/4
1987	729	55.8	7.1	31.2	8.7	4.6	8.0	10.7	5/5
1988	796	62.2	5.5	31.2	8.7	9.3	8.0	10.7	4/4
1989	796	55.0	4.8	31.2	8.5	6.2	8.0	10.0	4/5
1990	820	48.5	3.1	31.2	7.5	4.2	8.0	9.5	14/16
1991	820	59.1	2.8	31.2	6.5	2.3	8.0	9.0	13/15
1992	820	65.5	2.2	7.5	6.5	2.1	11.3	8.5	7/8

* Admt = Air dried metric tonne

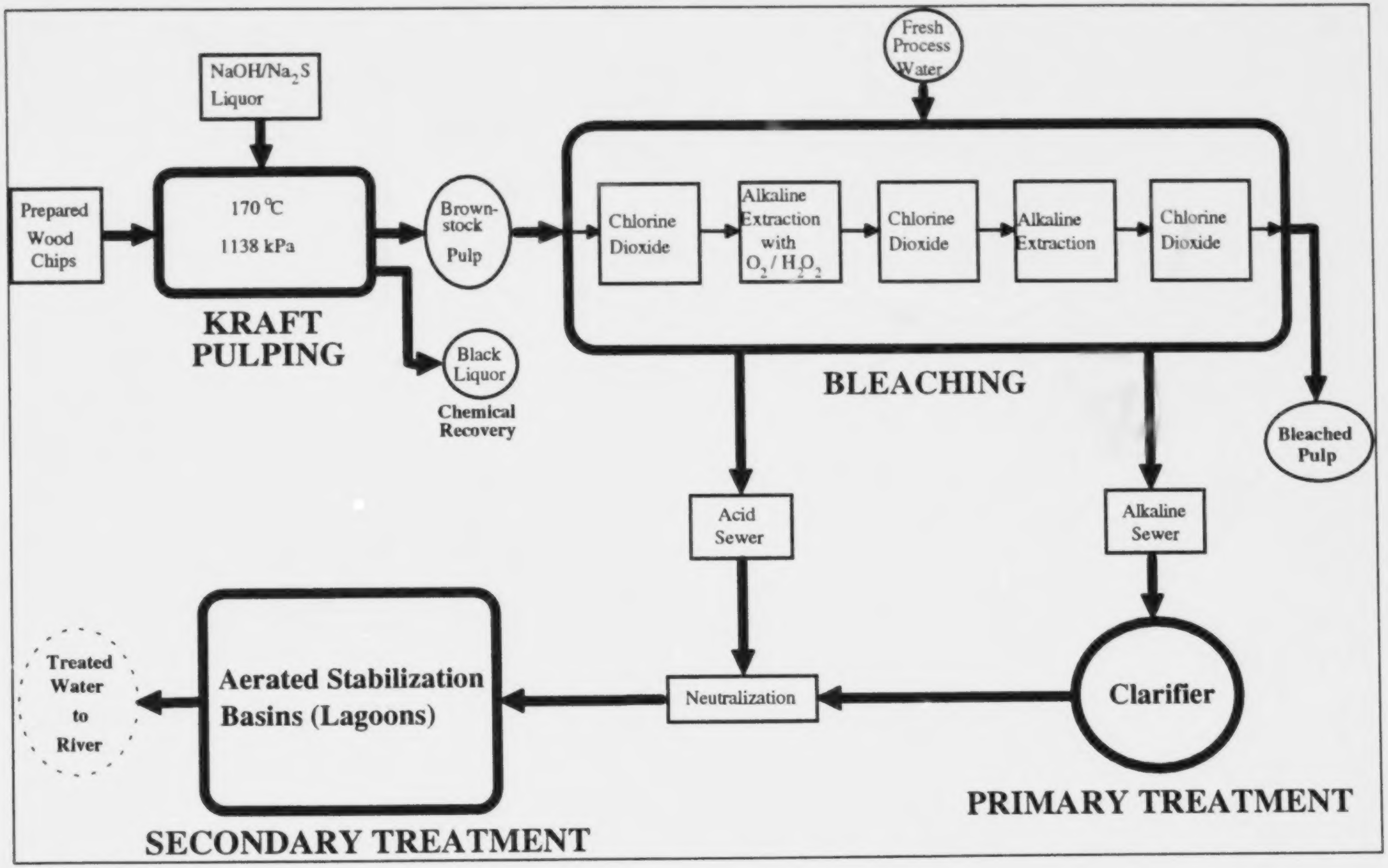
Prepared Wood Chips
NaOH/Na$_2$S Liquor
170 °C
1138 kPa
KRAFT PULPING
Brown-stock Pulp
Black Liquor
Chemical Recovery
Fresh Process Water
Chlorine Dioxide
Alkaline Extraction with O$_2$ / H$_2$O$_2$
Chlorine Dioxide
Alkaline Extraction
Chlorine Dioxide
BLEACHING
Bleached Pulp
Acid Sewer
Alkaline Sewer
Clarifier
Neutralization
PRIMARY TREATMENT
Aerated Stabilization Basins (Lagoons)
Treated Water to River
SECONDARY TREATMENT

Figure 1: Weyerhaeuser Pulp Mill pulping, bleaching and wastewater treatment processes (from information in Lindsay, 1993).

3.1.1 Pulping Process

The pulping process used in the Weyerhaeuser mill is the kraft process. The wood chips are digested under high temperature (170 °C) and pressure (1138 kPa), with NaOH liquor and Na_2S to dissolve the lignin and release the cellulose fibers (Lindsay, 1993).

3.1.2. Bleaching Process

The Weyerhaeuser mill uses 100% chlorine dioxide substitution in a five stage bleaching process as follows (Lindsay, 1993).

1. Chlorine dioxide bleaching
2. Alkaline extraction with oxygen and hydrogen peroxide
3. Chlorine dioxide bleaching
4. Alkaline extraction
5. Chlorine dioxide bleaching

After switching from 30% chlorine dioxide substitution in 1990 to 100% ClO_2 substitution in 1992 the AOX monthly average values dropped from 2.1 kg/adt to 0.6 kg/adt (Lindsay, 1993).

3.1.3. Wastewater Treatment Processes

3.1.3.1. Primary Treatment

The Weyerhaeuser mill uses a large circular clarifier that removes 95% total suspended solids and some BOD_5 (Lindsay, 1993). This effluent is then mixed with wastewater from the acid sewer to achieve neutralization (Lindsay, 1993).

3.1.3.2. Secondary Treatment

The Weyerhaeuser plant uses an aerated stabilization basin system for its secondary treatment (Lindsay, 1993). The BOD_5 reduction in this system is 95% (*Section 4.1.1 in this report*). Other changes in the effluent after the ASB are shown in Section 4.1.1 of this report. After leaving the lagoon the effluent flows into a foam pond, to increase the level of dissolved oxygen and dissipated foam (Lindsay, 1993). The effluent then flows into a discharge pipe buried ~1.8 meters in the Wapiti river bed (Lindsay, 1993). The ASB system is designed to treat the effluent for 10-14 days.

3.2. Wastewater Streams Characterization

3.2.1. Sampling

Procedures for the sample collection, handling, transport and storage were followed according to Standard Methods (1992) and Water Pollution Control Federation (1980).

Grab samples of the primary and secondary effluent were collected in 20 liter plastic pails with retractable pouring spouts. The pails of effluent were then shipped via courier to the Newton Research building whereupon they were stored at 4 °C in a walk-in refrigerator.

The primary and secondary effluents used in this study were collected on November 10, 1992, and April 20, 1993.

3.2.2. Analytical Procedures

The color analysis for the wastewaters and ozonated wastewaters was performed by H. Mao for the Weyerhaeuser project (Mao, Personal Communication, 1994). The method used to analyze the color was according to Standard Methods (1992), section 2120 B (2-2).

The chemical oxygen demand (COD) analysis was performed according to Standard Methods (1992) section 5220 (5-6). See appendix A for a detailed listing of the COD method used.

A Xertex Dohrman DC-80 TOC analyzer was used for the total organic carbon (TOC) analysis. The samples were analyzed according to the operations manual, with one exception. The instrument calibrates itself correlating the sample size with the standard concentration as follows: 1 µL - 10 ppm C; 200 µL - 400 ppm C ; 40 µL - 2000 ppm C. The reactor system was found to be an inefficient oxidation method for the pulp mill wastewaters, and, therefore, the furnace was used to completely oxidize the samples. Each sample analyzed was in the 0 to 400 ppm C range. In order to obtain more accurate results for the samples the volume was set at 200 µL so as to obtain a 400 ppm calibration, even though the volume used for the furnace had to be 40 µL. After much discussion and studies it was found that this method was correct in accuracy and precision. A summary of the TOC method is as follows:

1. The sample volume was set at 200 µL.
2. 40 µL of 400 ppm C potassium hydrogen phthalate (KHP) was added to the sample boat in the furnace tube. The KHP solution was made according to the operation manual.
3. The sample boat was then injected into the furnace and the analysis was started. The standard was run in triplicate.

4. The wastewater sample was then analyzed using 40 μL. The sample was run in duplicate. The total carbon of the sample was obtained in this way.

5. The total inorganic carbon (TIC) was analyzed by injecting 200 mL of the 400 ppm (KHP) standard into the reactor filled with potassium persulfate - mercuric salt solution (made according to the operation manual). The standards were again run in triplicate.

6. The UV lamp was turned off and 200 μL of the sample was injected in duplicate runs. The TIC of the sample was obtained in this way. 200 μL of 400 ppm C sodium carbonate (Na_2CO_3) was also injected and was found to be within 1% of 400 ppm C TIC. The Na_2CO_3 solution was also made according to the operation manual.

7. The TOC was then calculated by subtracting the TIC from the total carbon.

3.3. Ozone Reactor Systems and Analysis

3.3.1. Ozone Generator

A PCI Ozone Corporation ozone generator, model C2P-9C-4, was used in this project. The unit was operated in accordance with the manufacturer's instructions.

The ozone generator has two plate electrodes between which pure oxygen or air could flow. A high direct current voltage is applied across the electrodes in order to maintain a corona discharge (Bellamy et al., 1991). A corona discharge results during the collisions of air or oxygen molecules at high accelerations formed from the attractions to these highly charged plates (Halliday and Resnick, 1974). The air or oxygen molecules may even glow visibly due to light emitted during these collisions (Halliday and Resnick, 1974). Ozone is formed from these high velocity collisions of oxygen molecules (Bablon et al., 1991).

If air is used in ozone generation the resulting nitrogen oxides can form nitric acid, which can cause corrosion (Bablon et al., 1991). According to the operations manual oxygen gas can produce twice the amount of ozone as air. Due to these two facts compressed oxygen gas was used. In accordance with the operations manual the oxygen was extra dry and had a dew point of at least -50 °C. The dewpoint is a measure of the absolute moisture content of the oxygen gas and is used to denote the temperature at which moisture will condense from a flow of gas (Bellamy et al., 1991). A low moisture content is important in that water vapor will reduce the ozone generator efficiency (Bablon et al., 1991).

For safety purposes the ozone generator and the ozone reactors were located in a fume hood, during ozone experiments. This was important due to the high toxicity of ozone.

3.3.2. Ozone Reactors

The ozone reactor (reactor A) used for ozonation of the Nov./92 secondary effluent was as described elsewhere (Smith et al., 1991).

Reactor A (Figure 2) consists of two distinct parts, with the upper part a round bottom flask and he lower part a graduated cylinder for holding the effluent. The cylindrical part had a capacity of 0.4786 L and the volume of the spherical section was 1.078 L. the two parts were joined with a 6 mm thick walled T-joint. The third end of the T-Joint was connected to a teflon stopper valve, to be used as the gas inlet to the reactor. The neck part of the round bottom flask was a 24/40 ground glass joint with a teflon sleeve to prevent gas leakage. Springs were used to hold this joint together. The top of this joint had a teflon stopper valve attached to it, to be used as the gas outlet.

Ozone reactor B (Figure 3) was used for ozonation of the April/93 secondary effluent. It consisted of a cylindrical section with a volume of 1.940 L and a ground glass male ball joint at the top. The reactor had a sintered glass sparger in it for ozone diffusion into the wastewater. There was also a long glass rod with two flat propeller pieces on it and a stirring magnet enclosed in glass attached at the bottom. The top of this stirring rod was held in place by the top part of the reactor. A stirring plate was used to operate this stirring mechanism. Using a peristaltic pump the wastewater was pumped into the reactor. KI traps at the outlet end were used to measure the residual ozone and a wet test meter was attached at the outlet of these traps in order to keep the volume of the ozone/oxygen gas consistent from run to run.

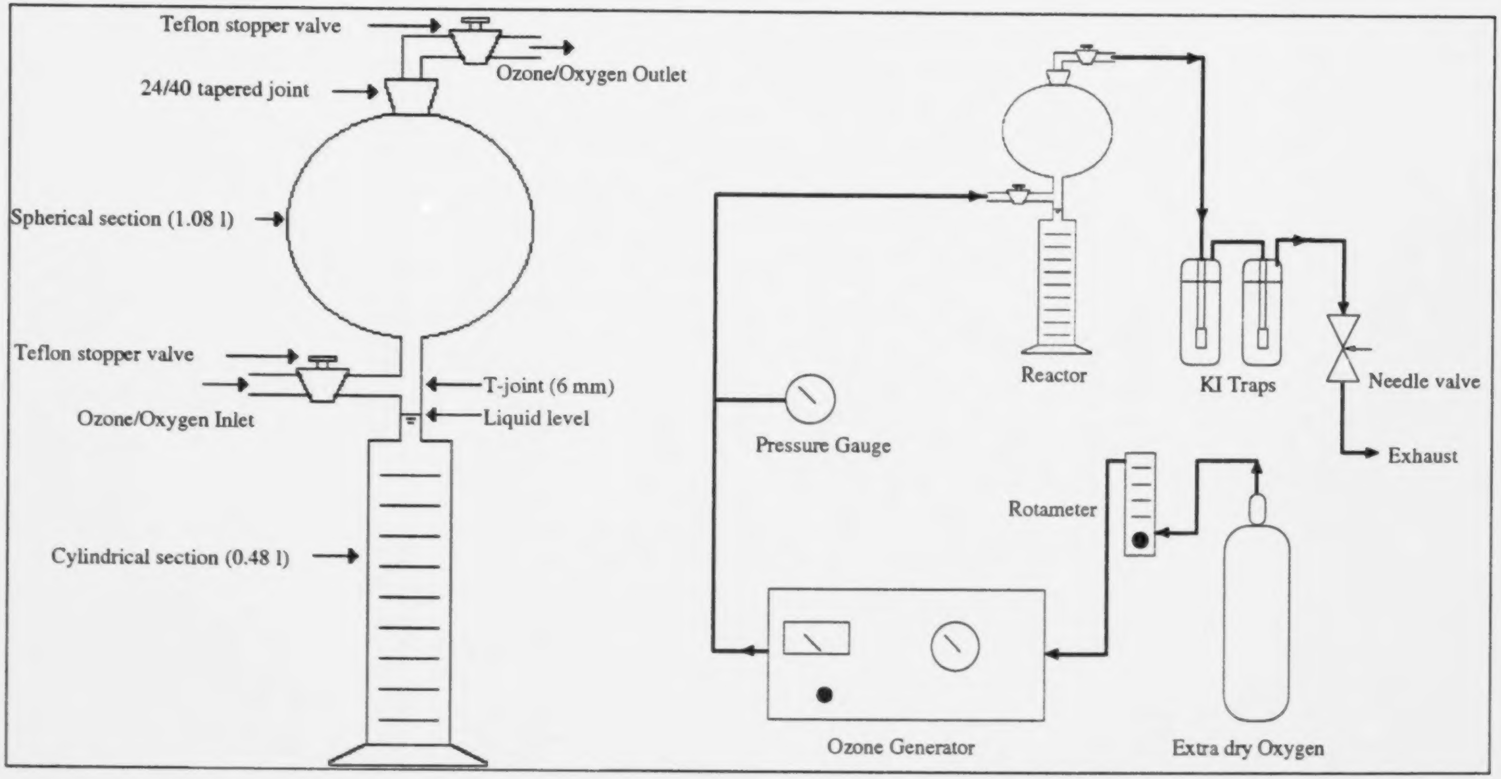
Teflon stopper valve
Ozone/Oxygen Outlet
24/40 tapered joint
Spherical section (1.08 l)
Teflon stopper valve
T-joint (6 mm)
Ozone/Oxygen Inlet
Liquid level
Cylindrical section (0.48 l)
Reactor
KI Traps
Needle valve
Exhaust
Pressure Gauge
Rotameter
Ozone Generator
Extra dry Oxygen

Figure 2: Reactor A and the Ozone System Equipment Set-up

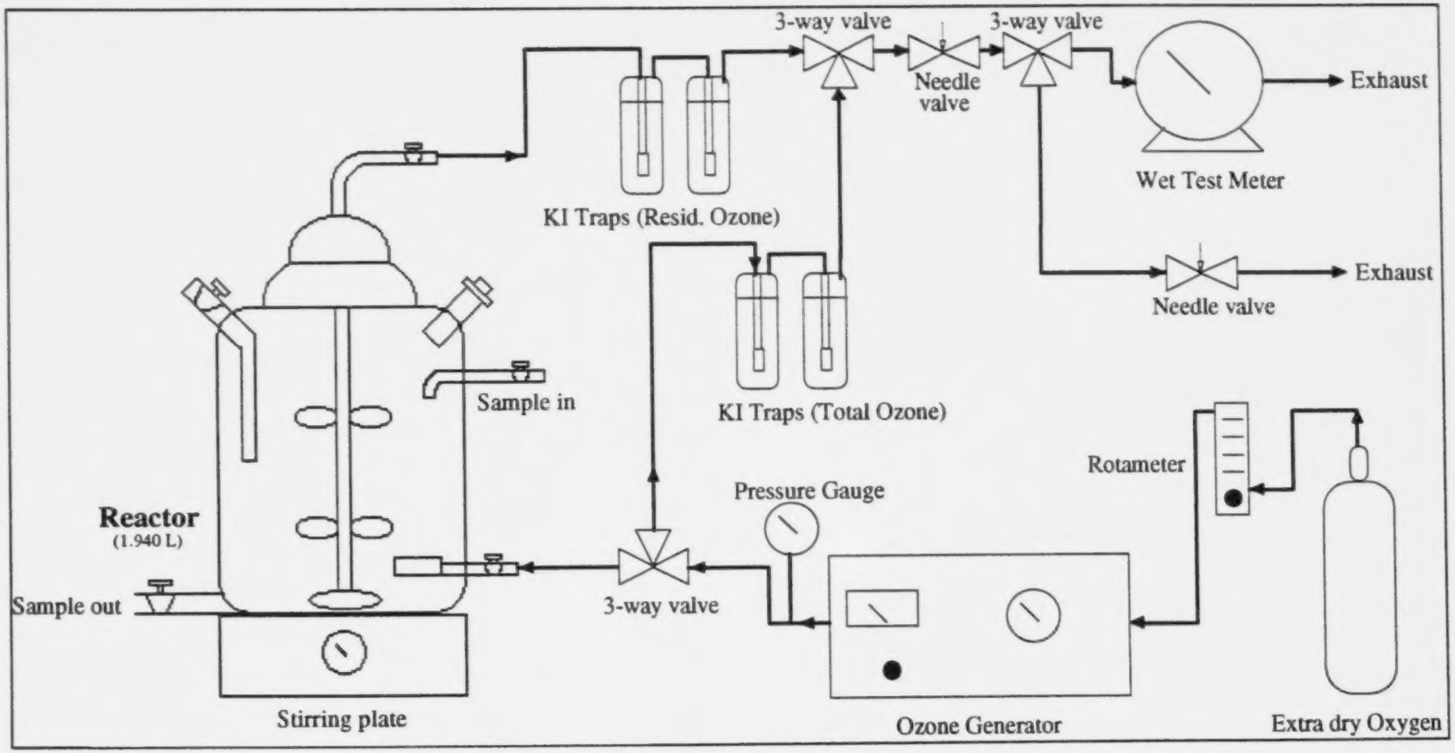
3-way valve
3-way valve
Needle valve
Exhaust
Wet Test Meter
KI Traps (Resid. Ozone)
Needle valve
Exhaust
Sample in
KI Traps (Total Ozone)
Rotameter
Pressure Gauge
Reactor
(1.940 L)
Sample out
3-way valve
Stirring plate
Ozone Generator
Extra dry Oxygen

Figure 3: Reactor B and the Ozone System Equipment Set-up

3.3.3. Total Ozone Measurement

For reactor A, 2 sparging tubes with ~200 mL of 2% KI solution (made with O.D.F.W.) were attached to the gas outlet of the reactor. Ozone demand-free water was added to the cylindrical portion of the reactor. The ozone/oxygen mixture was then flowed through the spherical section for 10 minutes after the pressure stabilized with the KI traps not connected. After 10 minutes the inlet and outlet values were closed and the KI traps were attached to the outlet. Prepurified nitrogen gas was then flowed through the reactor forcing the ozone/oxygen mixture into KI traps where it converted the iodide ion into iodine (brown color). The partially oxidized KI solution was then titrated with a standard solution of sodium thiosulfate and the total ozone was calculated (mL of 0.1000 M thiosulfate = 2.4 mg ozone (Standard methods, 1992)).

For reactor B, 2 sparging tubes with ~400 mL of 2% KI solution were used for the total ozone measurements. Two three-way valves at the outlet and inlet of the reactor could be positioned such that the ozone/oxygen mixture flowed through the reactor or was diverted to the KI traps. Using the gas meter to measure the same volume of ozone/oxygen through both avenues (usually 3 L) the amount of ozone was consistent. The KI solution was then titrated with sodium thiosulfate in order to measure the total ozone concentration.

3.3.4. Ozone Dose Measurement (Wastewater)

During the ozonation of the wastewater in reactor A the ozone/oxygen mixture was first flowed through the spherical section for 10 minutes while the wastewater was in the cylindrical section. Then the inlet and outlet valves were closed and the whole reactor was shaken by hand for 10 minutes to mix the wastewater with the ozone/oxygen gas. The outlet was then connected to the KI traps and N_2 flowed through the reactor forcing the residual ozone into the traps.

Experiments were undertaken with reactor A to determine if some of the ozone was decomposing during the mixing process (H. Mao, personal communication, 1994). These tests showed that 10.39% of the total ozone added decomposed. Therefore, the ozone dose or ozone consumption was calculated by subtracting both the residual ozone and the decomposed ozone from the total ozone, and then dividing by the wastewater volume (0.4786 L), as follows:

Ozone dose = [(total ozone)-(residual ozone)-(decomp. ozone)]/(0.4786 L)

For reactor B the ozone residual was measured by titrating the KI solution in the trap connected to the outlet of the reactor, filled with wastewater. The ozone dose was then calculated by subtracting the residual ozone from the total ozone and dividing that result by the wastewater volume (1.940 L), as follows:

Ozone dose = [(total ozone)-(residual ozone)]/(1.940 L)

3.4. Molecular Weight Distribution Method

3.4.1. Ultrafiltration of Mill Wastewaters and Ozonated wastewaters

The ultrafiltration of the secondary effluent B and the ozonated (84.8 mg/L) secondary effluent B was performed by H. Mao for the Weyerhaeuser project (Mao, personal communication, 1994). A Millipore Minitan acrylic ultrafiltration system was used with three low-binding regenerated cellulose membranes, which had molecular weight cut-offs of 10000, 5000 and 1000. The ultrafiltration method is detailed in Appendix A. After using this method five samples were obtained as follows:

1. >10,000 MWCO
2. 10,000 to 5,000 MWCO
3. 5,000 to 1,000 MWCO
4. <1,000 MWCO
5. wash water (also <1,000 MWCO)

The color, COD, TOC and MWD results for the <1,000 MWCO fraction and the wash water sample were combined using the following formula (TOC is used in the formula as an example):

TOC(combined) = TOC(<1,000) + [TOC(ww) x Volume(ww) / Volume(<1,000)]
where ww = wash water

After combining the results for these two samples there were four fractions designated as follows:

F1 = <1,000 MWCO

F2 = 5,000 to 1,000 MWCO

F3 = 10,000 to 5,000 MWCO

F4 = >10,000 MWCO

3.4.2. Size Exclusion HPLC

A flow diagram of the HPLC system used is shown in figure 4. The detector originally used was a Waters 484 UV-Vis single wavelength detector. The SEC data in this thesis was acquired using the diode array UV-Vis detector (DAD). The DAD could detect up to 8 wavelengths for each HPLC run. The data acquisition system was originally a Dionex 4400 integrator. In December of 1993 a new data acquisition system was purchased consisting of a Hewlett-Packard 35900 multichannel interface and a 486DX computer with Hewlett-Packard HPChem software. This software was specifically designed for HPLC work and could control the diode array detector. The signal data from the chromatograms was loaded into a Microsoft Excel worksheet, where calibration data could be used in order to obtain a molecular weight distribution.

A user's manual explaining the SEC method in detail, including instructions on the use of the HPChem software, is included in Appendix B.

3.4.2.1. Column

The analytical column used for size exclusion chromatography was the Waters Ultrahydrogel 250. The column dimensions were 7.8 mm diameter by 30 cm long. The column packing was cross-linked hydroxylated polymethacrylate gel with residual carboxyl groups. The manufacturer's estimated pore size for the packing in this column was 250 Å. The manufacturer's suggested efficiency was 14,000 theoretical plates and the suggested exclusion limit was 80,000 Da. The pH range was 2 - 12, which gave good versatility in the testing of various mobile phases. The manufacturer's suggested flow range was 0.5 to 0.8 mL/min, with a maximum of 1.0 mL/min.

A Waters Ultrahydrogel guard column was used at the inlet end of the analytical column in order to protect the analytical column from possible contamination during the analysis of the pulp mill wastewaters.

The guard and analytical column were cleaned approximately every two weeks of regular use. Each column was cleaned separately and the line to the detector was rerouted into a waste beaker so as to alleviate possible contamination of the detector. The cleaning procedure for each column was as follows:

1. 20% methanol / 80 % milli-Q water was pumped through the column at a flow rate of 0.5 mL/min for 1 hour.

2. Gradient run from 20% methanol / 80 % milli-Q water to 50% methanol / 50 % milli-Q water for 1 hour at a flow rate of 0.5 mL/min.

3. 50% methanol / 50 % milli-Q water was pumped through the column at a flow rate of 0.5 mL/min for 10 to 20 hours.

4. 100% milli-Q water was pumped through the column at a flow rate of 0.5 mL/min for 3 hours.

The methanol used was HPLC grade, which was filtered through a 0.1 µm membrane by the manufacturer. The milli-Q water was filtered through a 0.45 µm membrane filter (Millipore HVLP) to remove any particulate matter that would cause HPLC pump problems.

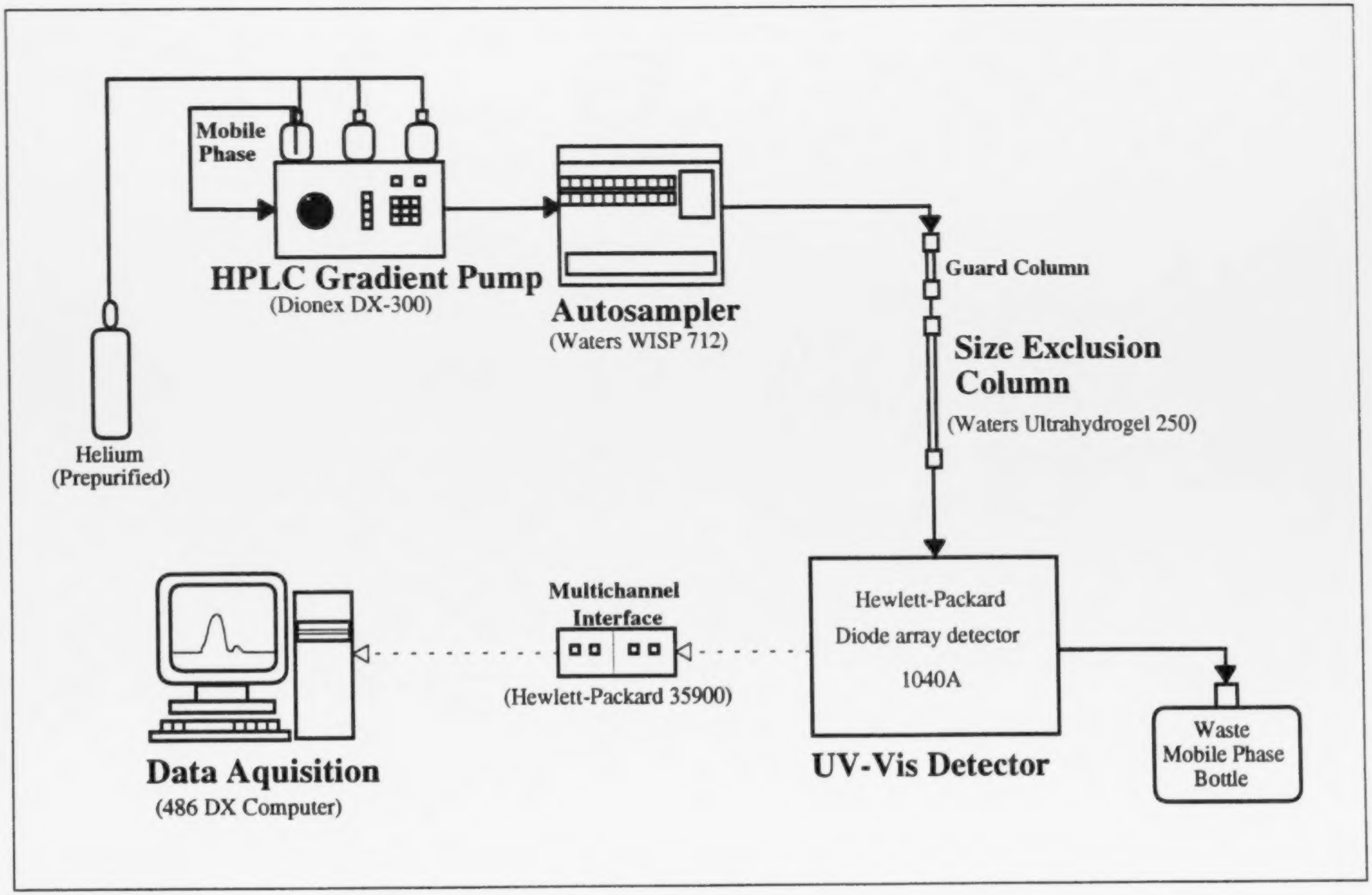
Mobile Phase
HPLC Gradient Pump
(Dionex DX-300)
Autosampler
(Waters WISP 712)
Guard Column
Size Exclusion Column
(Waters Ultrahydrogel 250)
Helium (Prepurified)
Multichannel Interface
(Hewlett-Packard 35900)
Hewlett-Packard
Diode array detector
1040A
UV-Vis Detector
Waste Mobile Phase Bottle
Data Aquisition
(486 DX Computer)

Figure 4: HPLC System Flow Diagram

3.4.2.2. Mobile Phase

The mobile phase testing results are shown in section Appendix C. The mobile phase selected was 25 mM carbonate buffer with a pH of 10.3. It was prepared as follows:

1. 2.650 grams Na_2CO_3 and 2.100 grams $NaHCO_3$ (each weighed accurately) was added to a 2 liter volumetric flask and diluted to the mark with milli-Q H_2O.
2. The mobile phase was then filtered through a 0.45 μm membrane filter (Millipore HVLP).

This mobile phase was degassed by flowing Helium gas (prepurified) through it for 10 minutes.

3.4.2.3. Sample Preparation

The pulp mill wastewaters, ozonated wastewaters and ultrafiltration fractions were prepared for the SEC runs as follows:

1. A 50 mM Carbonate Buffer was made:

   2.650 grams Na_2CO_3 and 2.100 grams $NaHCO_3$ (weighed accurately) were added to a 1 liter volumetric flask and diluted to the mark with milli-Q H_2O. This solution was filtered through a 0.45 μm membrane filter (Millipore HVLP).

2. The samples were then filtered through a 0.45 μm membrane filter (Millipore HVLP).
3. The samples were then diluted 1:1 with the 50 mM carbonate buffer solution (i.e., 1000 μL wastewater and 1000 μL 50mM carbonate buffer).

3.4.2.4. Molecular Weight Calibration Standards

For the final MWD calibrations the following standards were used:

1. Polyethylene glycols

   MW = 194, 600, 4100 and 12600 Da

2. Pullulan or polytrisaccharides

   MW = 5800, 12200, 23700, 48000 Da

3. Peptides with the following formulas:

i)	Ac - [KLEALEA]- amide	MW =	814
ii)	Ac - $[KLEALEA]_2$- amide		1569
iii)	Ac - $[KLEALEA]_4$- amide		3079
iv)	$[Ac - [KLEALEA]_4$- amide$]_2$		6158

Each set of standard solutions were prepared as follows:

Pullulans (Pull) - 0.02 g (20 mg) of each Pullulan were added to 1.000 mL of 25 mM carbonate buffer to make ~2% solutions. Each Pullulan standard solution was run separately.

Polyethylene Glycols (PEG) - 20 mg of PEG 600, 4100 and 12600 were each added to 1.000 mL of 25 mM carbonate buffer to make ~2% solutions. 30 mg of PEG 194 was added to 1.000 mL of 25 mM carbonate buffer to make an ~3% solution. Equal volumes of these four standard solutions were then mixed together (i.e., 500 µL of each into a separate vial). The four PEG standards were then run as a mixture.

Peptides Mixture (Pept) - The peptides were received from the Protein Engineering department of the Heritage research center as a mixture in milli-Q H_2O. This solution was diluted 1:1 with the 50 mM carbonate buffer solution (i.e., 1000 µL peptide mixture and 1000 µL 50mM carbonate buffer). The peptide standards were then run as a mixture.

Typical chromatograms and integration results are shown in Appendix E for each set of standards.

3.4.2.5. Detection Wavelengths

The detection wavelength used for the SEC analysis of the samples was 225 nm. For the peptide standards a detection wavelength of 225 nm was also used. For the PEG and Pullulan standards 245 nm was used as the detection wavelength. With the use of the diode array detector up to 7 wavelengths could be monitored during each run. Therefore, both required wavelengths could be monitored during a set of runs.

The method used to select the detection wavelengths for the samples and standards is described in Appendix C

3.4.2.6. Flow

The operating flow rate change recommended by the manufacturer for the column was 0.5 mL/min to 0.8 mL/min, with the maximum being 1.0 mL/min. Operating within the suggested limits the change in flow rate had very little impact on the resolution. 0.5 mL/min was used as the flow for the majority of the development runs because it gave a slightly wider MWD for the sample chromatograms.

0.8 mL/min was chosen as the flow rate for the final set of SEC analysis because the calibration linearity and the resolution were very similar to the 0.5 mL/min flow rate. The faster flow rate meant a decrease in the analysis time from 30 minute (0.5 mL/min) to 20 minutes (0.8 mL/min).

A flow rate test was done on the system using a volumetric burette and a stop watch. At a setting of 0.5 mL/min the measured flows in triplicate were 0.500, 0.501 and 0.501. These results indicate the flow rate setting on the pump is very accurate.

4. RESULTS

4.1. Characterization of Pulp Mill Wastewater Streams

4.1.1. Primary and Secondary Effluents

Primary and Secondary Effluents collected on November 10, 1992, (effluent A) and April 20, 1993, (effluent B) were analyzed for color, COD, TOC and BOD_5. The Results are shown in Table 3.

Table 3: Characteristics of Primary and Secondary Effluents

Parameter	Nov/92 Primary Effluent	Nov/92 Secondary Effluent	Apr/93 Primary Effluent	Apr/93 Secondary Effluent
Color (c.u.)	1650	1444	1430	1390
COD (mg/L)	1368	810	1439	676.8
TOC (mg/L)	493	276.3	444*	247*
BOD_5 [1]	460	25.1	333.9*	12.9*

* These are results for 0.8 μm filtered samples.

4.1.2. Secondary Effluent Ultrafiltration Fractions

The Apr/93 Secondary Effluent was ultrafiltered using the method described in Appendix A of this report (H. Mao, personal communication, 1994). The ultrafiltered fractions were analyzed for Color, COD TOC and BOD_5. The results of these analysis are shown in table 4.

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[1] BOD_5 was performed by H. Mao for the Weyerhaeuser project (Mao, Personal Communication, 1994)

Table 4: Characteristics of Ultrafiltration Fractions from Secondary Effluent (% of total in brackets)

Parameter	F4 (> 10 kDa)	F3 (10 - 5 kDa)	F2 (5 - 1 kDa)	F1 (< 1 kDa)	Total
Color (c.u.)	885 *(69.7)*	187 (14.7)	104 (8.2)	94 (7.4)	1270
COD (mg/L)	306 (48.3)	111 (17.5)	84 (13.3)	132 (20.9)	633
TOC (mg/L)	113 (45.7)	46 (18.6)	36 (14.6)	52 (21.1)	247
BOD_5 [2]	6.6 (24.6)	4.2 (15.7)	3.8 (14.2)	12.2 (45.5)	26.8

4.2. Ozonation of Secondary Effluent

For this report Secondary effluent was ozonated with three doses: two with effluent A and one with effluent B. The two ozone doses used on effluent A were achieved using reactor A and the one ozone dose used on effluent B were achieved using reactor B. Each ozone dose was run in duplicate. R1 & R2 and R4 & R5 were the duplicates ran using reactor A and NR1 & NR2 were the duplicates using reactor B. The total ozone was measured in triplicate for the two ozone runs on effluent A and in quadruplicate for the ozone run on effluent B. The methods used for the determination of total, residual and ozone dose are described in sections 3.3.3 and 3.3.4 of this report. The ozone dose was then calculated according to section 3.3.4.

Table 7 shows the ozone run results for the 3 ASB effluent samples. A t-test was performed to determine whether the results from the duplicate ozone runs could be pooled together. The calculated t-values are all less than the tabulated t-values at a confidence level of 95% (Miller et al., 1990), therefore the results from the duplicate ozone dose runs can be pooled together. For each parameter tested such as COD, TOC, and MWDs the results will be averaged for the duplicate ozone dose samples.

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[2] BOD_5 was performed by H. Mao for the Weyerhaeuser project (Mao, Personal Communication, 1994)

Table 5: Ozone Run Results and t-tests for the determination of pooling duplicates

Sample R1					Sample R2			
O3 Total (mg)	**O3 Total - decomp O3 (mg)**	**O3 Resid. (mg)**	**O3 Dose (mg/l)**		**O3 Total (mg)**	**O3 Total - decomp O3 (mg)**	**O3 Resid. (mg)**	**O3 Dose (mg/l)**
58.32	52.26	0.73	108.08		58.32	52.26	1.75	105.94
62.66	56.15	0.73	116.23		62.66	56.15	1.75	114.09
60.70	54.39	0.73	112.55		60.70	54.39	1.75	110.41
Mean			**112.29**					**110.15**
Std dev			**4.08**					**4.08**
n			**3**					**3**

t-calc **0.641**
t(4) @ 95% **2.776**

Sample R4					Sample R5			
O3 Total (mg)	**O3 Total - decomp O3 (mg)**	**O3 Resid. (mg)**	**O3 Dose (mg/l)**		**O3 Total (mg)**	**O3 Total - decomp O3 (mg)**	**O3 Resid. (mg)**	**O3 Dose (mg/l)**
105.12	94.20	2.26	192.82		105.12	94.20	1.07	195.32
102.86	92.17	2.26	188.58		102.86	92.17	1.07	191.07
104.76	93.88	2.26	192.15		104.76	93.88	1.07	194.64
Mean			**191.18**					**193.68**
Std dev			**2.28**					**2.28**
n			**3**					**3**

t-calc **1.339**
t(4) @ 95% **2.776**

Sample NR1					Sample NR2			
O3 Total (mg)	**O3 Total - decomp O3 (mg)**	**O3 Resid. (mg)**	**O3 Dose (mg/l)**		**O3 Total (mg)**	**O3 Total - decomp O3 (mg)**	**O3 Resid. (mg)**	**O3 Dose (mg/l)**
177.20	177.20	15.57	83.31		177.20	177.20	14.41	83.91
181.14	181.14	15.57	85.35		181.14	181.14	14.41	85.94
181.86	181.86	15.57	85.72		181.86	181.86	14.41	86.31
177.44	177.44	15.57	83.44		177.44	177.44	14.41	84.04
Mean			**84.45**					**85.05**
Std dev			**1.29**					**1.29**
n			**4**					**4**

t-calc **0.654**
t(6) @ 95% **2.447**

A summary of the total, residual and dosage for each ozone run are shown in table 6.

Table 6: Summary of the Ozone doses applied to Secondary Effluents

Sample	**Reactor**	**Volume of effluent (mL)**	**Total Ozone (mg)**	**Residual Ozone (mg)**	**Decomp. Ozone (mg)**	**Ozone Dose (mg/L)**	**O3 Dose std dev**
Effluent A	A	476.8	60.6	1.2	6.3	**111.2**	*4.1*
Effluent A	A	476.8	104.2	1.7	10.8	**192.4**	*2.3*
Effluent B	B	1940	179.4	15.0	n/a	**84.8**	*1.3*

n/a - not applicable

4.2.1. Chemical Oxygen Demand

The COD analysis was performed using the method described in Appendix A of this report. Each sample was run in duplicate. A summary of the COD results is listed in table 7.

Table 7: COD Analysis Results for the Ozonated Effluents, Ultrafiltration Fractions of the 84.8 mg O_3/L Ozonated Effluent B and the Original Effluents

Sample (ozone dose)	**COD (mg/L)**	**ΔCOD *(%Δ COD)** (mg/L)**
84.8	606.6	70.2 *(-10.4)*
192.8	628.0	182 *(-22.5)*
F4 of 84.8 mg O_3/L Eff B	232.2	73.8 *(-24.1)*
F3 of 84.8 mg O_3/L Eff B	98.9	12.1 *(-10.9)*
F2 of 84.8 mg O_3/L Eff B	80.2	3.8 *(-4.5)*
F1 of 84.8 mg O_3/L Eff B	165.7	+33.7 *(+25.5)*
effluent A	810.0	--------
effluent B	676.8	--------

* The original ultrafiltration fraction COD data is shown in Table 4

4.2.2. Total Organic Carbon

The TOC analysis was performed using the method described in section 3.2.2. of this report. Each sample was run in duplicate. A summary of the TOC results is listed in table 10.

Table 8: TOC Analysis Results for the Ozonated Effluents and Original Effluents

Sample (ozone dose)	**TOC (mg/L)**	**ΔTOC *(%Δ TOC)** (mg/L)**
84.8	236.5**	10.5 *(-4.3)*
111.6	263.3	13.0 *(-4.7)*
192.8	258.7	17.6 *(-6.4)*
F4 of 84.8 mg O_3/L Eff B	91.2	21.8 *(-19.3)*
F3 of 84.8 mg O_3/L Eff B	40.2	5.8 *(-12.6)*
F2 of 84.8 mg O_3/L Eff B	33.5	2.5 *(-6.9)*
F1 of 84.8 mg O_3/L Eff B	70.4	+18.4 *(+35.4)*
effluent A	276.3	----------
effluent B	247**	----------

* The original ultrafiltration fraction TOC data is shown in Table 4

** These are results for 0.8 µm filtered samples.

4.3. Molecular Weight Distributions

The conditions used for the SEC work are as outlined in section 3.4.2. Samples were run in triplicate. Baseline corrections were carried out on each of the chromatograms, due to slight deviations from zero and drift in the baseline. The points were taken at 6 minutes, retention time, 5 on each side and the 6 minute absorbance. These 11 absorbencies were then averaged and this value along with 6 minutes retention time was used as the first point in the baseline. Another 11 points were averaged at 15 minute retention time to comprise the second point in the baseline. This baseline was subtracted from the chromatogram, in order to zero the non-signal baseline and also to connect for drift.

4.3.1. Molecular Weight Distribution Calibrations

The SEC calibrations were undertaken using the standards and procedures outlined in section 3.4.2.4 and. The standards were run in triplicate. Typical standard chromatograms are shown in appendix D of this report. Table 9 lists the SEC calibration data and the calibration plot is shown in figure 5.

Table 9: SEC Calibration Data

std type	*mol wt*	*log(MW)*	*ret time (min)*	*rt std dev*
pullulan	48000	4.681	7.842	0.014
pullulan	23700	4.375	8.455	0.014
pullulan	12200	4.086	9.061	0.021
pullulan	5800	3.763	9.770	0.008
PEG	12600	4.100	8.556	0.046
PEG	4100	3.613	9.814	0.053
PEG	600	2.778	11.560	0.047
PEG	194	2.288	13.429	0.050
peptide	6158	3.789	8.578	0.006
peptide	3079	3.488	9.685	0.011
peptide	1569	3.196	10.347	0.011
peptide	814	2.911	11.606	0.071

The linear regression results are as follows:

Std Type	**Formula**	**R^2**
pullulan	log(mol wt) = 8.404 - 0.476 x (ret time)	1.000
PEG	log(mol wt) = 7.329 - 0.381 x (ret time)	0.986
peptide	log(mol wt) = 6.330 - 0.297 x (ret time)	0.987
ALL	**log(mol wt) = 7.696 - 0.415 x (ret time)**	**0.930**

Vapor Pressure Osmometry (VPO) was attempted on the ultrafiltration fractions so as to try to obtain an average molecular weight of these fractions. These average molecular weights were to be used as further calibration information. Unfortunately, this method was not successful. The VPO results are shown in Appendix C.

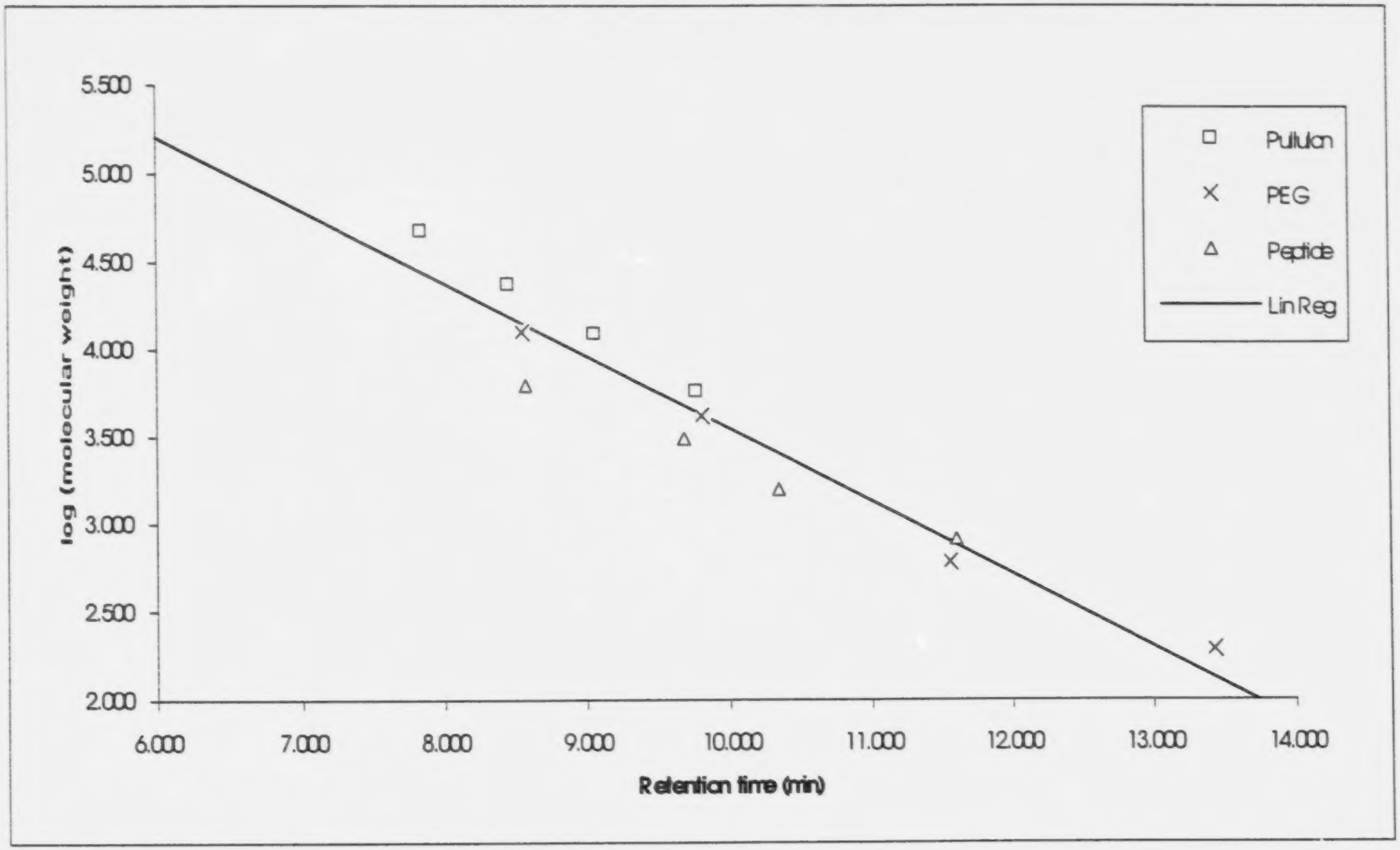

Figure 5: SEC Calibration Plot

4.3.2. Primary Effluent (ASB Influent)

The primary effluent MWD's are shown in figures 6 and 7. The numerical characterization data for the MWDs of the ASB Influents are shown in tables 10 and 11. The distribution areas were determined by computer integration of the chromatograms using a peak width of 0.3 and a threshold of -4. The weighted average molecular weights were determined by first calculating the weighted average retention time (t_w) as follows:

$$t_w = \frac{\sum_{i=1}^{k} a_i t_i}{\sum_{i=1}^{k} a_i}$$

where,
t_i = time
a_i = absorbance at time t_i

k = number of observations or signals

The weighted average molecular weights (WAMW) were then calculated by substituting the value for t_w into the linear regression formula determined from the calibration results as follows:

$$\text{WAMW} = 10^{(7.696 - 0.415(t_w))}$$

The number average molecular weight was determined by first calculating the number average retention time using the following formula:

$$t_n = \frac{\sum_{i=1}^{k} t_i}{k}$$

where,
t_i = time
k = number of observations or signals

The number average molecular weights (NAMW) were then calculated by substituting the value for t_n into the linear regression formula determined from the calibration results as follows:

$$\text{NAMW} = 10^{(7.696 - 0.415(t_n))}$$

The dispersivity, which is a measure of the skewness of the MWDs, was calculated by dividing the number average molecular weight into the weighted average molecular weight. The molecular weight ranges were determined from the retention times at 1% and 99% of $\sum a_i t_i$. One percent was used in order to alleviate any small baseline variations. It was determined that by using a percentage of the start of the deviation from zero the numbers were more consistent than choosing to use the actual retention times obtained from the baseline numerical deviation. When 1% was cut off from each tail the calculated high and low range values were very close to those which would be obtained by visual inspection. For example, in figure 6 visual inspection gives a range of ~22000 to ~200, which is very close to the range of 21341 to 249 obtained via the numerical calculation.

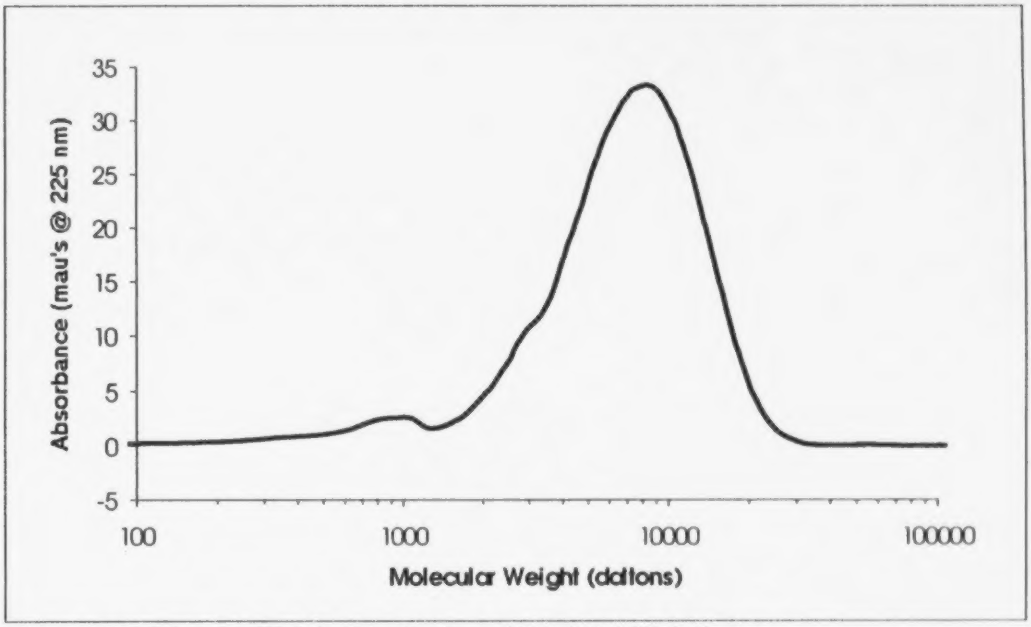

Figure 6: Molecular Weight Distribution of Nov/92 ASB Influent

Table 10: MWD characterization data for ASB Influent (11/92)

	Run #1	Run #2	Run #3	Mean	std. dev.
Distribution Area	3144	3170	3150	**3155**	*14*
Weighted Average Molecular Weight	6452	6582	5569	**6201**	*551*
Number Average Molecular Weight	1325	1597	1992	**1638**	*335*
Dispersivity	4.87	4.12	2.80	**3.93**	*1.05*
MW Range: High	22513	22502	19008	**21341**	*2020*
Low	219	238	291	**249**	*37*

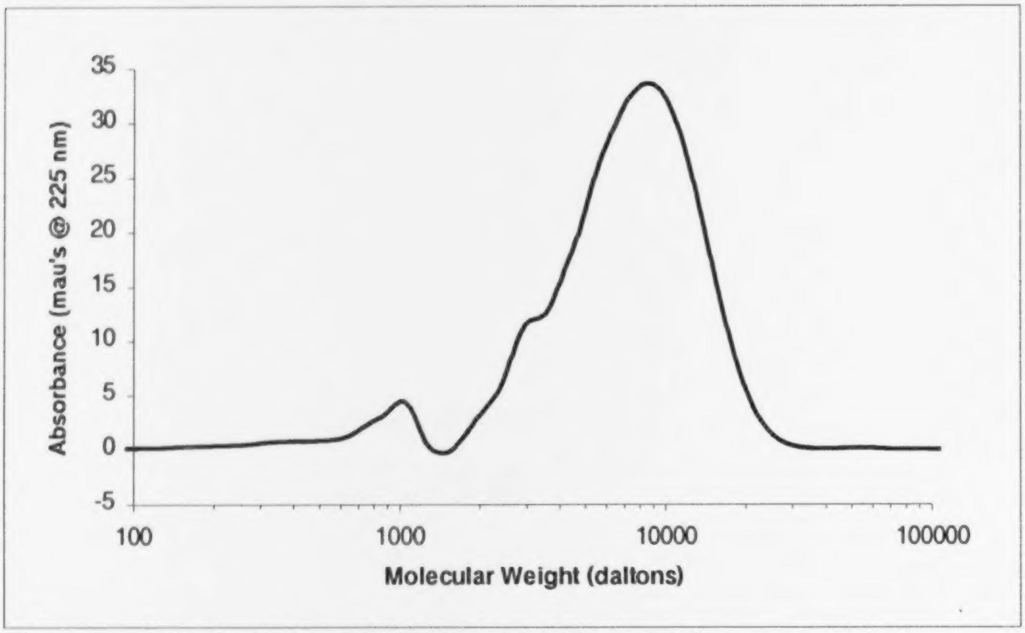

Figure 7: Molecular Weight Distribution of Apr/93 ASB Influent

Table 11: MWD characterization data for ASB Influent (04/93)

	Run #1	Run #2	Run #3	Mean	std. dev.
Distribution Area	3090	3163	3303	**3185**	*108*
Weighted Average Molecular Weight	6350	6392	6347	**6363**	*25*
Number Average Molecular Weight	1122	1431	1533	**1362**	*214*
Dispersivity	5.66	4.47	4.14	**4.76**	*0.80*
MW Range:					
High	21370	21435	22093	**21633**	*400*
Low	292	244	267	**268**	*24*

4.3.3. Secondary Effluent (ASB Effluent)

The ASB Effluent MWDs are shown in figures 8 and 9 along with the ASB Influent MWDs for comparison purposes. The numerical characterization data for the MWDs of the ASB Effluents are shown in tables 12 and 13.

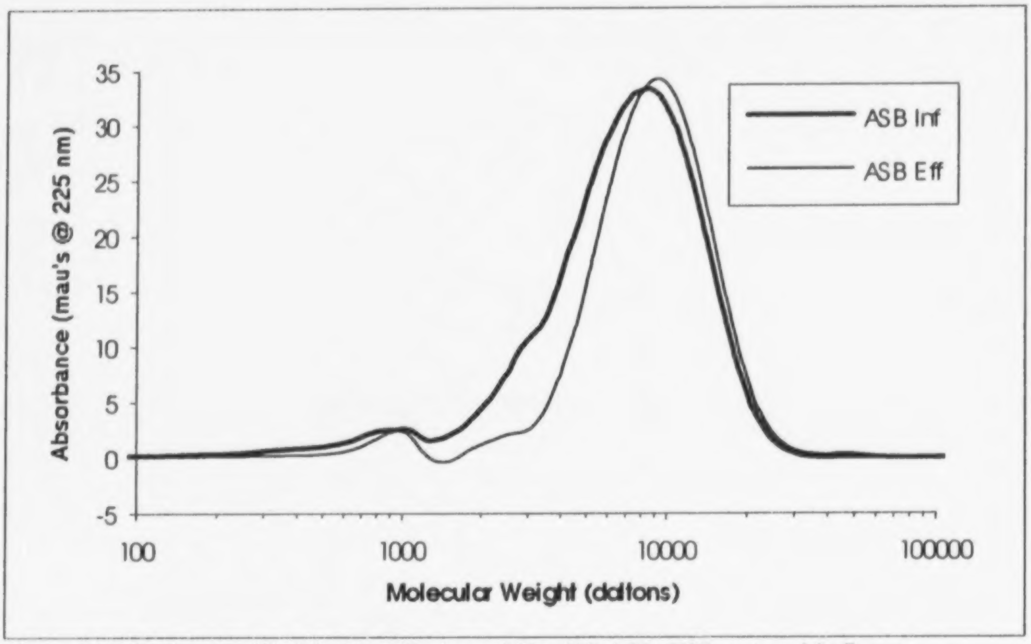

Figure 8: Molecular Weight Distribution of Nov/92 ASB Effluent and Influent

Table 12: MWD characterization data for ASB Effluent (11/92)

	Run #1	Run #2	Run #3	Mean	std. dev.
Distribution Area	2767	2769	2714	**2750**	*31*
Weighted Average Molecular Weight	8240	8384	7384	**8002**	*541*
Number Average Molecular Weight	1723	1760	1769	**1751**	*25*
Dispersivity	4.78	4.76	4.17	**4.57**	*0.35*
MW Range:					
High	25811	25253	22744	**24603**	*1634*
Low	360	595	512	**489**	*119*

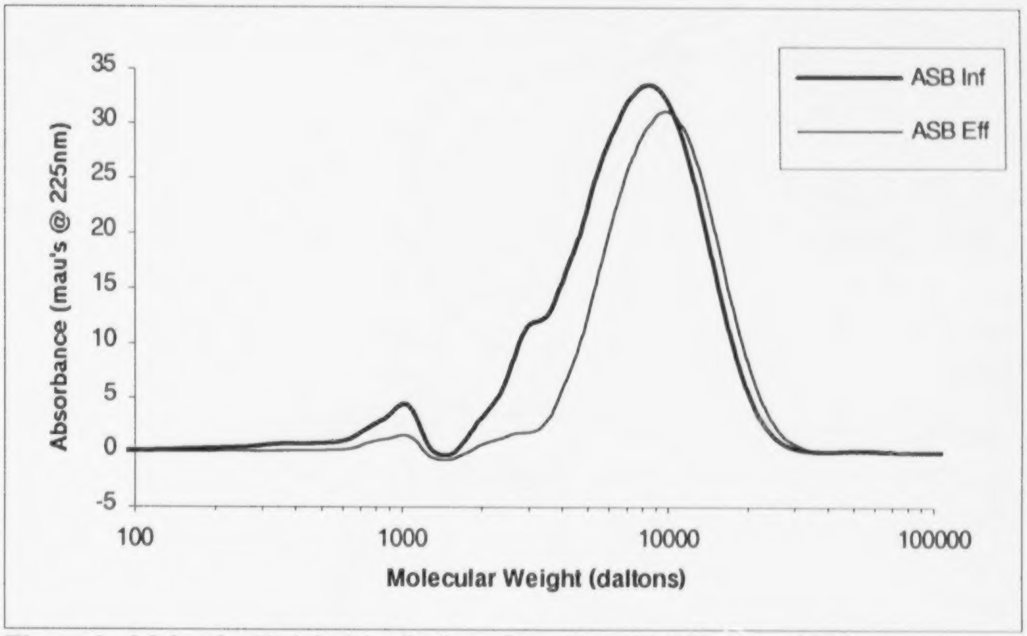

Figure 9: Molecular Weight Distribution of Apr/93 ASB Effluent and Influent

Table 13: MWD characterization data for ASB Effluent (04/93)

	Run #1	Run #2	Run #3	Mean	std. dev.
Distribution Area	2540	2499	2542	**2527**	*24*
Weighted Average Molecular Weight	8716	8563	10231	**9170**	*922*
Number Average Molecular Weight	1571	1416	2999	**1995**	*873*
Dispersivity	5.55	6.05	3.41	**5.00**	*1.40*
MW Range: **High**	24759	24182	30804	**26582**	*3668*
Low	746	482	719	**649**	*145*

4.3.4. Secondary Effluent Ultrafiltration Fractions

The MWDs of the Ultrafiltration fractions from the Apr/93 Secondary Effluent are shown in figure 10 along with the ASB Effluent B MWD. Table 14 lists the numerical characterization data for the MWDs of each fraction. Figure 11 compares the original ASB Effluent MWD with the Sum of the UF MWDs.

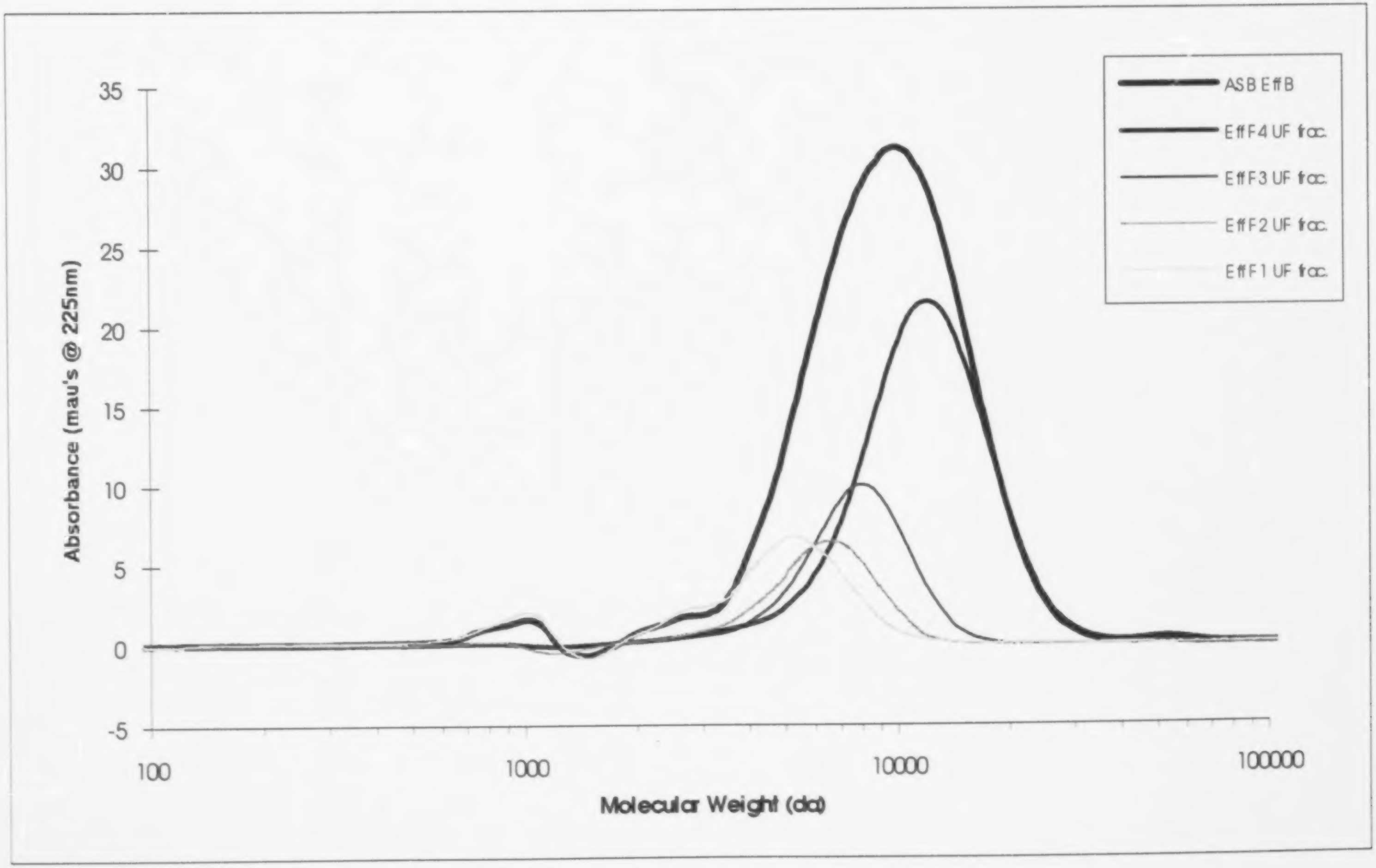

Figure 10: Molecular Weight Distributions of Ultrafiltration Fractions from ASB Effluent B and the original Effluent B

Table 14: MWD characterization data for the Ultrafiltration fractions from the ASB Effluent (04/93)

	Distribution Area	**WAMW**	**NAMW**	**Dispersivity**	**MW Range**	
					High	**Low**
F4						
#1	1317	11655	11138	1.05	29952	3051
#2	1366	11594	12430	0.93	35793	2740
#3	1321	11403	12967	0.88	30501	2806
Mean	**1335**	**11551**	**12178**	**0.95**	**32082**	**2866**
std dev	*27*	*131*	*940*	*0.09*	*3225*	*164*
F3						
#1	497	7504	5329	1.41	14735	2590
#2	501	7526	5657	1.33	15092	2457
#3	511	7344	5393	1.36	14877	2302
Mean	**503**	**7458**	**5460**	**1.37**	**14901**	**2450**
std dev	*7*	*99*	*174*	*0.04*	*179*	*144*
F2						
#1	376	5853	1987	2.9	11767	688
#2	396	5756	1433	4.0	11660	409
#3	416	5722	2455	2.3	12150	582
Mean	**396**	**5777**	**1958**	**3.1**	**11859**	**560**
std dev	***20***	***67***	***512***	***0.9***	***257***	***141***
F1						
#1	534	3840	1547	2.5	11168	224
#2	532	3274	1916	1.7	14940	153
#3	523	3873	2116	1.8	9724	589
Mean	**530**	**3663**	**1859**	**2.0**	**11944**	**322**
std dev	***6***	***337***	***288***	***0.4***	***2693***	***234***

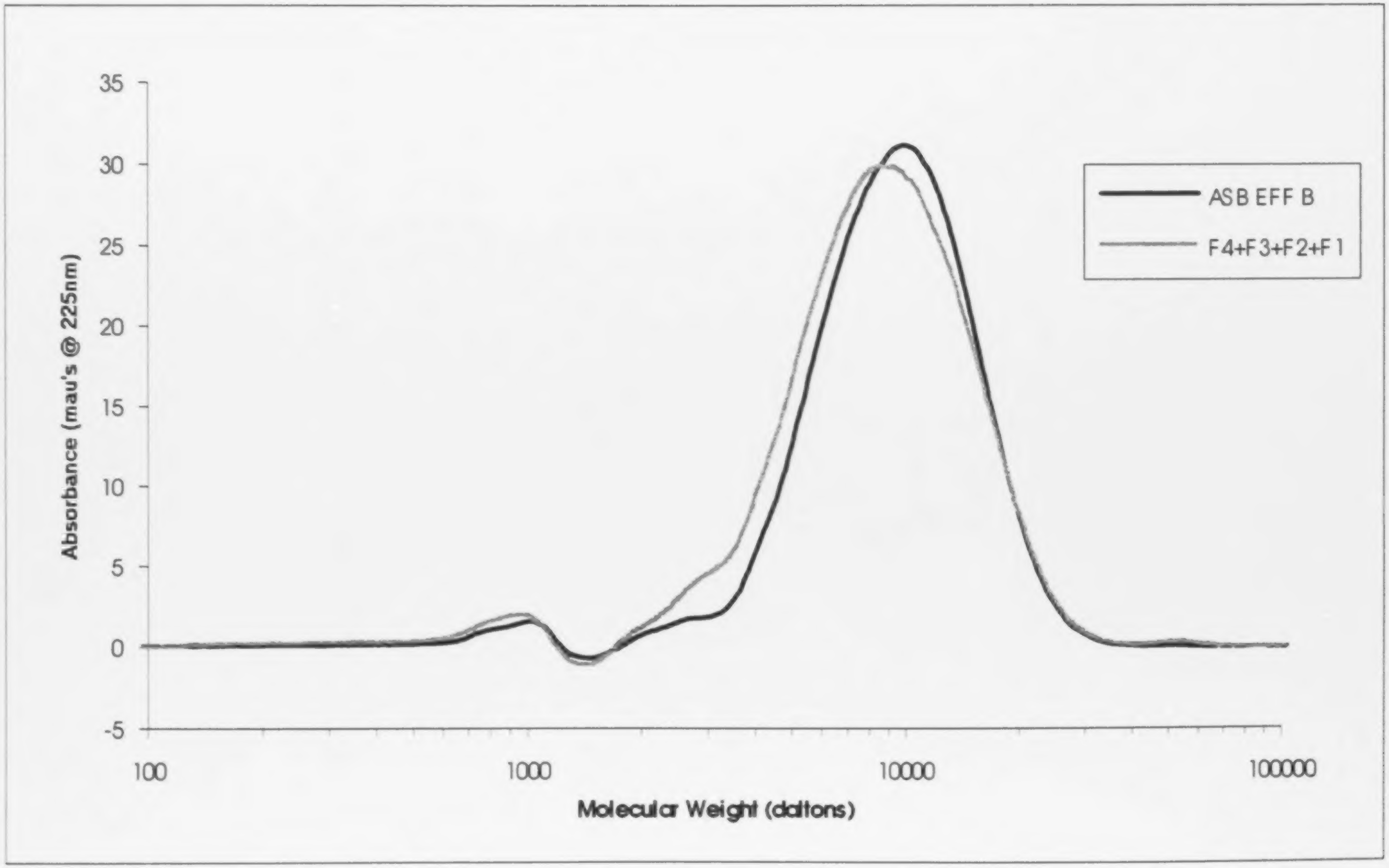

Figure 11: Comparison of ASB Effluent B MWD with the Sum of the Ultrafiltration fractions MWD

4.3.5. Ozonated Secondary Effluent

The ozonated ASB Effluent MWDs along with the original Effluent MWDs are shown in figures 12 and 13. The numerical characterization data for the MWDs of the Ozonated ASB Effluents are shown in tables 15, 16 and 17.

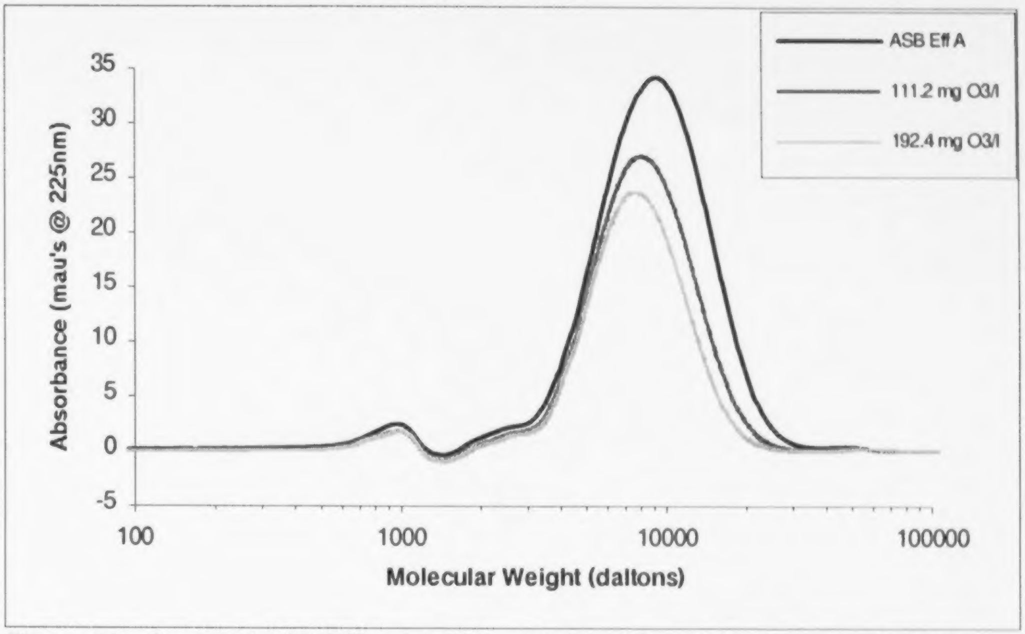

Figure 12: Ozonated ASB Effluent MWDs and the original ASB Effluent A MWD

Table 15: MWD characterization data for 111.2 mg O_3/L Ozonated ASB Effluent

	R1 #1	R1 #2	R1 #3	R2 #1	R2 #2	R3 #3	Mean	std. dev.
Distribution Area	2013	1947	2108	2052	2077	2093	**2048**	*60*
Weighted Average Molecular Weight	7775	7552	6964	7598	7551	7122	**7427**	*312*
Number Average Molecular Weight	4035	2239	2543	2433	2304	2115	**2612**	*713*
Dispersivity	1.9	3.4	2.7	3.1	3.3	3.4	**3.0**	*0.6*
MW Range: High	21672	22409	21312	22168	22252	21441	**21876**	*460*
Low	772	374	495	625	446	508	**537**	*142*

Table 16: MWD characterization data for 192.4 mg O_3/L Ozonated ASB Effluent

	R4 #1	**R4 #2**	**R4 #3**	**R5 #1**	**R5 #2**	**R5 #3**	**Mean**	**std. dev.**
Distribution Area	1790	1753	1815	1667	1697	1665	**1731**	*64*
Weighted Average Molecular Weight	7118	7102	6812	7029	7079	6915	**7009**	*121*
Number Average Molecular Weight	1349	1523	1271	1327	1595	1920	**1498**	*241*
Dispersivity	5.3	4.7	5.4	5.3	4.4	3.6	**4.8**	*0.7*
MW Range: High	18800	19014	18530	18497	18972	18075	**18648**	*354*
Low	637	628	411	666	646	647	**606**	*96*

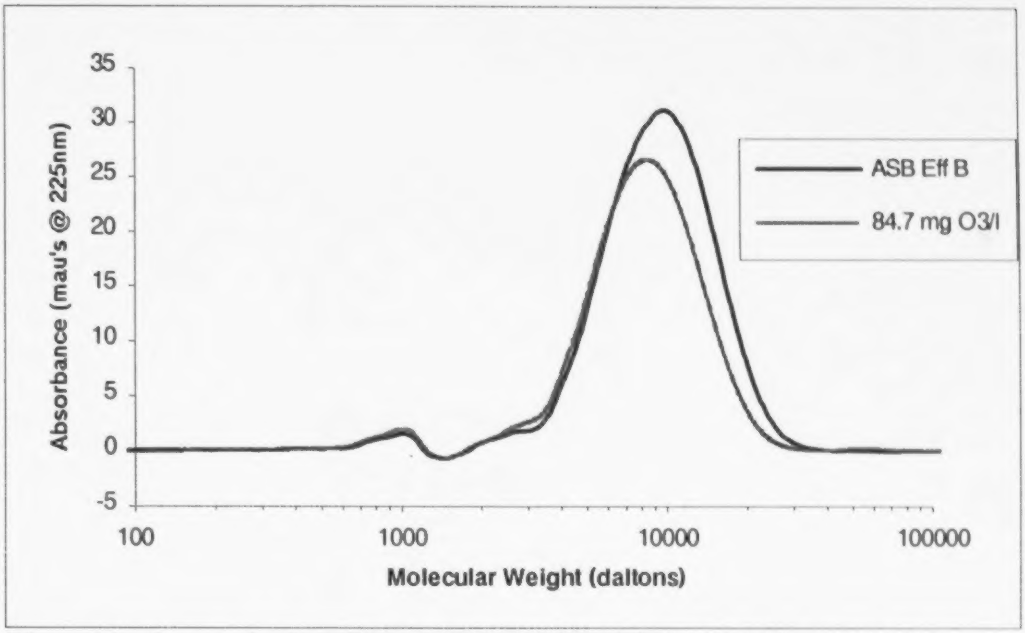

Figure 13: Ozonated ASB Effluent MWD and the original ASB Effluent B MWD

Table 17: MWD characterization data for 84.8 mg O_3/l Ozonated ASB Effluent

	NR1#1	NR1#2	NR1#3	NR2#1	NR2#2	NR2#3	Mean	std. dev.
Distribution Area	2180	2108	2090	2108	2185	2138	**2135**	*40*
Weighted Average Molecular Weight	7346	7393	6884	7916	7912	7857	**7551**	*417*
Number Average Molecular Weight	2526	1450	3035	2368	4160	3128	**2778**	*905*
Dispersivity	2.9	5.1	2.3	3.3	1.9	2.5	**3.0**	*1.1*
MW Range: High	21217	21575	24029	21634	25610	22434	**22750**	*1726*
Low	748	544	218	787	682	729	**618**	*213*

4.3.6. Ozonated Secondary Effluent Ultrafiltration Fractions

The duplicate runs from the 84.8 mg O_3/L Ozonated ASB effluent were each ultrafiltered according to the method outlined in Appendix A. The MWDs for each fraction of the ozonated effluent along with the original ASB effluent fraction MWDs are shown in figures 14 to 17. The MWD characterization data for each fraction of the ozonated effluent are shown in tables 18 to 21.

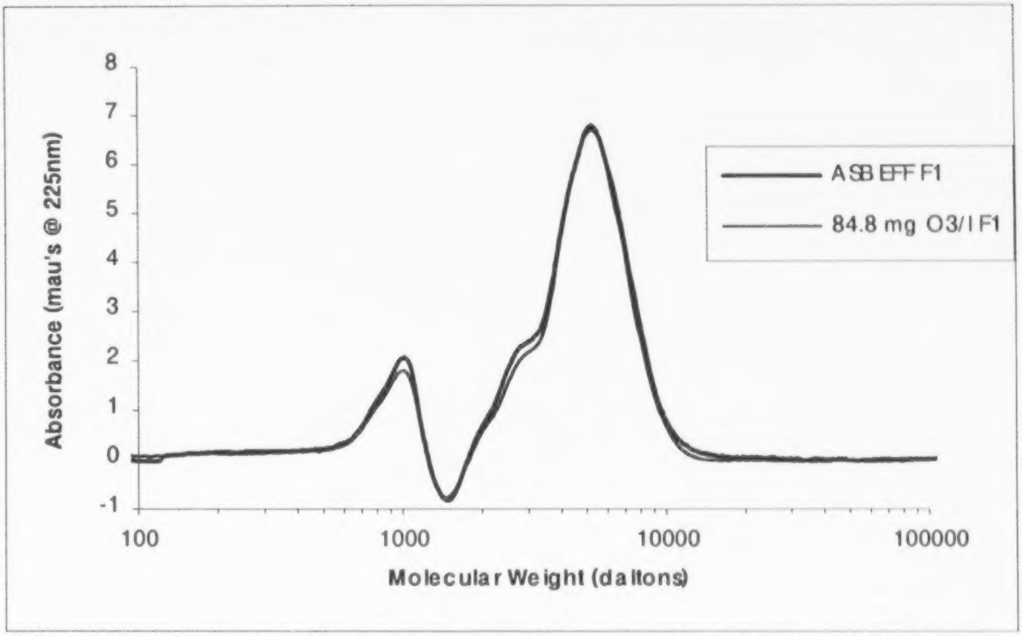

Figure 14: F1 fraction of Ozonated ASB Effluent (84.8 mg O_3/L) and F1 fraction of Original ASB Effluent B

Table 18: MWD characterization data for F1 fraction of Ozonated ASB Effluent (84.8 mg O_3/L)

	NR1#1	**NR1#2**	**NR1#3**	**NR2#1**	**NR2#2**	**NR2#3**	**Mean**	**std. dev.**
Distribution Area	510	539	527	507	523	474	**513**	*23*
Weighted Average Molecular Weight	3729	3707	3669	3976	3763	3772	**3769**	*108*
Number Average Molecular Weight	820	856	3691	2050	846	1643	**1651**	*1122*
Dispersivity	4.5	4.3	1.0	1.9	4.4	2.3	**3.1**	*1.5*
MW Range: High	10094	10098	24945	9899	9438	9784	**12376**	*6162*
Low	163	159	297	618	153	481	**312**	*197*

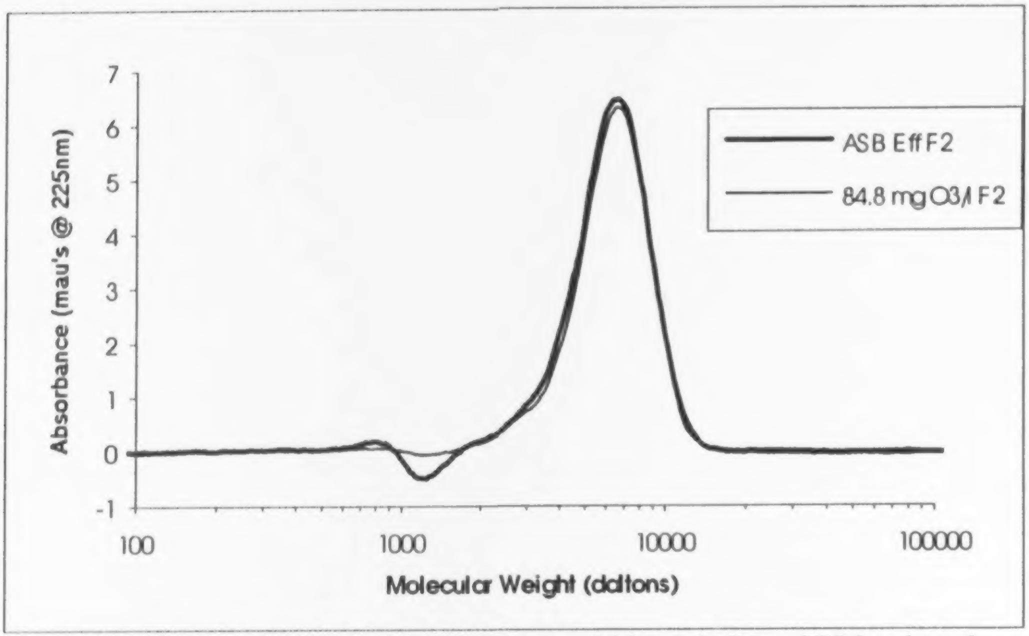

Figure 15: F2 fraction of Ozonated ASB Effluent (84.8 mg O_3/L) and F2 fraction of Original ASB Effluent B

Table 19: MWD characterization data for F2 fraction of Ozonated ASB Effluent (84.8 mg O_3/L)

	NR1#1	NR1#2	NR1#3	NR2#1	NR2#2	NR2#3	Mean	std. dev.
Distribution Area	323	312	312	321	293	325	**314**	*12*
Weighted Average Molecular Weight	6123	6115	5969	6247	6380	6101	**6156**	*141*
Number Average Molecular Weight	4784	4491	5217	4918	5369	4937	**4952**	*312*
Dispersivity	1.3	1.4	1.1	1.3	1.2	1.2	**1.2**	*0.1*
MW Range: **High**	12581	12487	12452	12214	11259	12134	**12188**	*486*
Low	2069	1962	1986	2259	2930	2177	**2231**	*361*

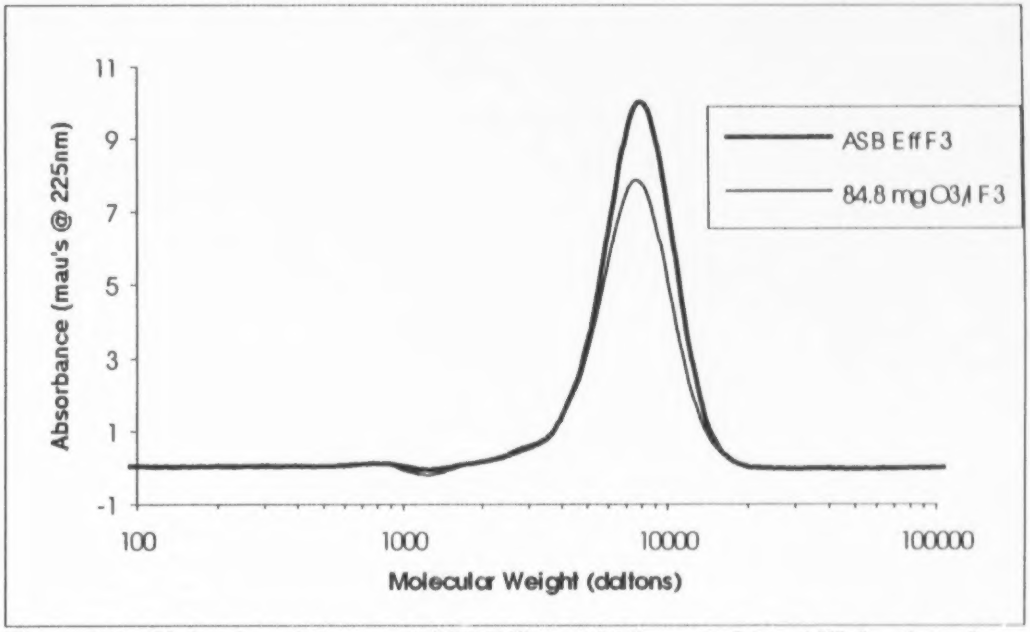

Figure 16: F3 fraction of Ozonated ASB Effluent (84.8 mg O_3/L) and F3 fraction of Original ASB Effluent B

Table 20: MWD characterization data for F3 fraction of Ozonated ASB Effluent (84.8 mg O_3/L)

	NR1#1	NR1#2	NR1#3	NR2#1	NR2#2	NR2#3	Mean	std. dev.
Distribution Area	406	441	445	419	458	430	**433**	*19*
Weighted Average Molecular Weight	7137	7280	7061	7328	7120	7191	**7186**	*101*
Number Average Molecular Weight	4847	5860	6251	5874	6332	9183	**6391**	*1466*
Dispersivity	1.5	1.2	1.1	1.2	1.1	0.8	**1.2**	*0.2*
MW Range: High	14526	15030	15022	14915	17070	16800	**15560**	*1084*
Low	2202	2536	2384	2662	1748	2300	**2305**	*319*

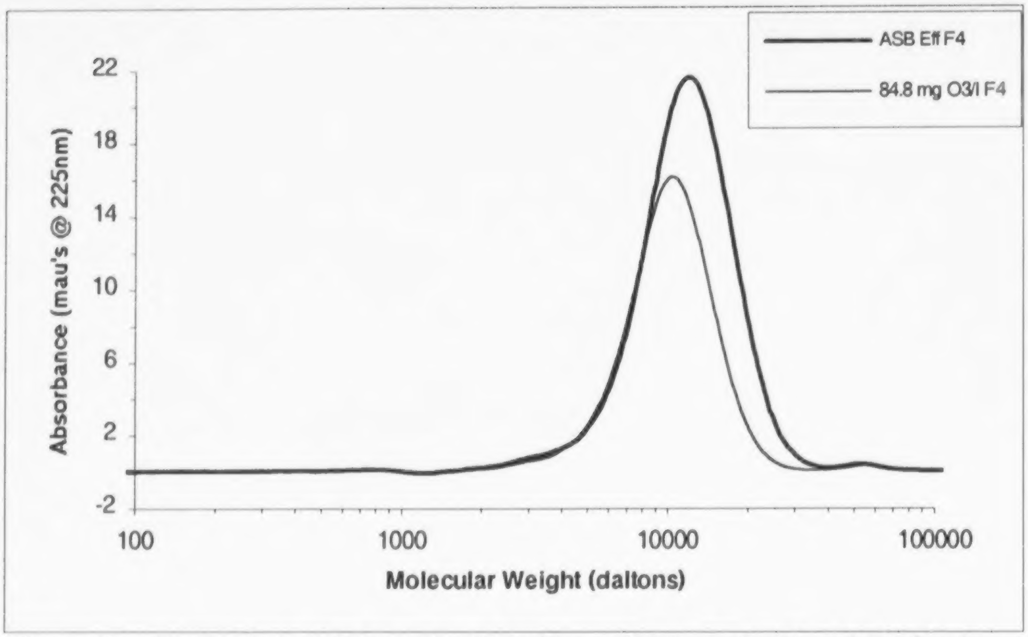

Figure 17: F4 fraction of Ozonated ASB Effluent (84.8 mg O_3/L) and F4 fraction of Original ASB Effluent

Table 21: MWD characterization data for F4 fraction of Ozonated ASB Effluent (84.8 mg O_3/L)

	NR1#1	NR1#2	NR1#3	NR2#1	NR2#2	NR2#3	Mean	std. dev.
Distribution Area	881	913	929	955	975	979	**939**	*38*
Weighted Average Molecular Weight	10044	10091	9794	10082	10211	9980	**10034**	*140*
Number Average Molecular Weight	10527	12768	11539	12359	12702	13870	**12295**	*1146*
Dispersivity	1.0	0.8	0.8	0.8	0.8	0.7	**0.8**	*0.1*
MW Range: High	24352	27074	37767	28085	45768	42022	**34178**	*8864*
Low	3073	2917	2638	2817	2729	2789	**2827**	*152*

4.3.7. Statistical Comparisons of MWD Data

In order to study the differences or similarities of the molecular weight distributions obtained in the study these differences or similarities must be proven by statistical analysis. The molecular weight distributions are log-normal or very close to log normal. Therefore, the MWD characterization data populations could not be assumed to be normal, and a nonparametric test was more appropriate for this data. The Mann-Whitney rank-sum test was used to compare the means, for each set of data, at a probability of 95% (Conover, 1980). The results of these tests are shown in Appendix D.

5. DISCUSSION

5.1. Molecular Weight Distribution Calibration

The calibration data shown in section 4.3.1 shows that the three types of standards give very similar retention times for equivalent molecular weights. The correlation coefficients are excellent for each individual set of standards and the R^2 value for all three sets of standards combined (12 compounds) is quite good (0.93). This is an indication that the buffer, flow rate and column give molecular size exclusion results that correlate very well to molecular weight exclusion results. Using a combination of standards with different chemical natures also helps to minimize specific problems associated with using one type of standard, which would be hard to recognize if problems such as adsorption and hydrophobic interactions were inherent in each polymeric unit.

5.2. Secondary Treatment

The BOD_5 reduction from the primary to secondary effluents was 95% (460 to 25.1 mg/L). This result indicates that the aerated stabilization basin is operating very efficiently, as 95% BOD_5 is at the upper range for ASBs (80 to 95% in McAllen,1989). COD and TOC reductions in the ASB are not quite as high as the BOD_5 reduction. This is expected, due to the predominance of larger lignin molecules, which are not readily biodegradable. In the lagoon, these molecules have large contributions to the total COD and TOC. The COD/TOC ratios are 2.9 and 2.7 for the two effluent samples. These ratios are characteristic of industrial wastewaters.

5.2.1 Molecular Weight Distributions

In looking at figures 8 and 9 along with the distribution area results in tables 10 to 13, there was an apparent loss of organic material when the primary effluent was treated in the aerated stabilization basin (ASB). The distribution area loss was 12.8% and 20.9% for influents A and B, respectively. However, the actual loss of organic material as represented by TOC in table 5, was 44.0% and 44.4% for influents A and B, respectively. This indicates that there were molecules in the ASB influent that have low absorptivities at a wavelength of 225 nm. The ASB influent contains some biodegradable compounds such as alcohols, sugars and carbohydrates. These smaller molecules tend to be saturated and saturated organics absorb mainly in the vacuum ultraviolet region, or at wavelengths below 200 nm (Parikh, 1974). This would account for the lower absorptivity of the ASB influent at 225 nm and hence the lower molecular weight distribution area. It would not be possible to alleviate this problem by detecting in the vacuum ultraviolet region due to the strong absorbance of water in this region.

The increase in weighted average molecular weight from the ASB influent to the ASB effluent is as would be expected. The biological degradation of smaller organic molecules in the lagoon results in a predominance of larger, less biodegradable, molecules in the effluent. The ASB influent biodegradable molecules which are seen by the UV-detector are smaller unsaturated or aromatic molecules that could be the result of lignin degradation. The increase in the high molecular range value from the primary to secondary effluent could be due to the addition of larger organic molecules, from cellular degradation of the lagoon organisms.

5.3. Ultrafiltration fractions

5.3.1. Color

As shown in table 4, the F1 fraction had the lowest color value of the four ultrafiltration fractions. The F1 fraction had 7.4% of the total color even though its TOC value was 21.1% of the total. The color values increased with increasing molecular weight fractions. Similar color results for ultrafiltration fractions of spent bleaching liquors were obtained by Hardell and de Sousa (1977) and also Pfister and Sjostrom (1978).

Hardell and deSousa (1977) ultrafiltered spent liquors from the chlorine and alkali extraction stages of a kraft process. Four fractions were obtained using 25000, 10000 and 1000 molecular weight cut-off membranes. For both spent liquors the color was lowest in the <1000 fraction. In the spent chlorination liquor this low molecular weight fraction contained 60% of the organic material (TOC), but only 20% of the total color. In the spent alkali extraction liquor this fraction contained 20% of the organic material (TOC), but only 1.5% of the total color. With both spent liquors the 25000 to 10000 fraction had the highest color value, with the >2500 and the 10000 to 1000 having the next two highest color values, respectively.

Pfister and Sjostrom (1978) bleached pine kraft pulp in a laboratory using conventional and oxygen bleaching sequences. Each of these spent bleaching liquors were then ultrafiltered into three fractions using 10000 and 1000 molecular weight cut-off membranes. Typical of all the liquors was that most of the color originated from the two higher molecular weight fractions of >10000 and 10000 to 1000. Correspondingly, the lowest fraction of <1000 gave the lowest color values. The low molecular weight fractions had from 2 to 40% of the total color and 8 to 60% of the dissolved organic material.

The color vs. Molecular weight relationships found in these literature studies and this study were as expected. The brown coloration, typical of kraft mill effluent, is primarily attributable to the presence of high molecular weight, lignin based compounds (Alberta Environment, 1992). High molecular mass materials in spent chlorination and alkali extraction liquors are the primary carriers of chromophoric structures that cause bleaching plant effluents to impart light-absorbing qualities to receiving waters (Kringstad and Lindstrom, 1984).

5.3.2. Biochemical Oxygen Demand

The F1 fraction had the highest BOD_5 value of all the fractions, as shown in table 4. The BOD_5 was 12.2 mg/L for this fraction, which corresponds to 45.5% of the total. However, the TOC for F1 was only 21.1% of the total. In the Hardell and deSousa

(1977) study the low molecular weight fraction (<1000) from the alkali extraction liquor contributed ~50% of the seven day BOD. In the Pfister and Sjostrom paper the low molecular weight fractions (<1000) from the chlorination, hypochlorination and oxygen spent liquors contributed the majority of seven day BOD to the total. These results are as expected, due to the lower molecular weight compounds being more biodegradable. The high molecular weight fractions are less biologically active, probably due to low penetration of the cell membranes of natural occurring bacteria (Kringstad and Lindstrom, 1984).

5.3.3 Molecular Weight Distributions

In looking at the molecular weight ranges and the weighted average molecular weights of the ultrafiltration fractions it is apparent that the manufacturer's membrane molecular weight cut-offs of 1000, 5000 and 10000 daltons are not very accurate in terms of this pulp mill ASB effluent. These so-called "cut-offs" are upper molecular weight limits for solute passage, designated by the membrane manufacturer, above which less than 5 or 10% solute passage occurs (Aptel and Clifton, 1986). The cut-offs are given in daltons and are reported for purified proteins due to their monodispersivity (Aptel and Clifton, 1986). On the basis of the cut-off number it is quite impossible to predict the ability of a membrane to filter out a given solute from an aqueous solution (Aptel and Clifton, 1986). This is because the cut-off is not an intrinsic property of the membrane, as the rejection coefficient depends on concentration polarization, the chemical nature of the macromolecule and the conformational changes in the macrosolute (Aptel and Clifton, 1986). This sample dependent nature of the ultrafiltration membrane performance was shown in two other studies. Becher et al. (1985) ultrafiltered chlorinated natural humic water and found that the molecular weight of the <1000 fraction's size exclusion peak was 7900 daltons. Pellinen and Salkinoja-Salonen (1985a) found that size exclusion results indicated that the <5000 ultrafiltration fraction of spent bleaching liquor had an upper range of 6310 daltons and that the >5000 fraction had a lower range of 158 daltons.

In looking at the ultrafiltration fractions a correlation can be made between the weighted average molecular weight and the color/TOC ratio of each fraction. This relationship is shown in figure 18. As discussed earlier, the increase in color with increasing molecular weight in the secondary effluent is as expected. It is, however, quite interesting to note that there is a straight line relationship between the WAMW and the color/TOC ratio. The location of the ASB effluent point on the line is as expected, because the ultrafiltration fractions are derived from the ASB effluent. The location of the ASB influent on the line indicates that the color causing molecules are not changed during secondary treatment.

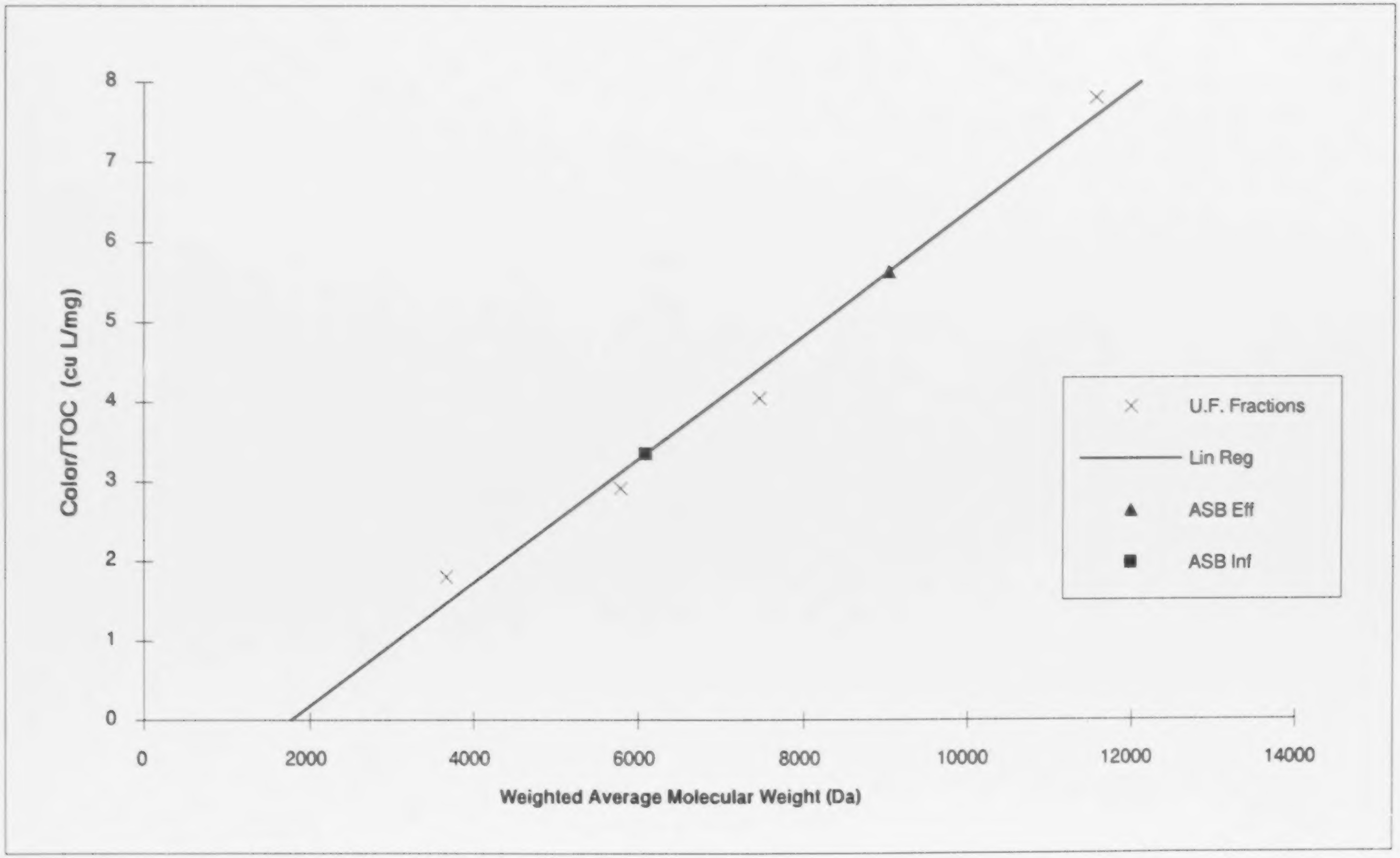

Figure 18: Color/TOC vs Weighted Average Molecular Weight for the Ultrafiltration Fraction ($R^2 = 0.98$) and the points for ASB Influent (04/93) and ASB Effluent (04/93)

5.4 Secondary Effluent Ozonation

As shown in section 4.2 the duplicate ozone doses on each effluent (A & B) could be pooled together. Therefore, the data characterizing each duplicate such as COD, color, TOC and molecular weight distributions were pooled together.

The relative standard deviations ranged from 3.7% (111.2 mg O_3 /L) to 1.2% (192.4 mg O_3/L for the ozone dose determinations. For reaction A the highest ozone dose standard deviation was 4.1 mg/L. This correlates very well to the variations found by Smith et al. (1991). Using the identical reactor they found that a maximum variation of 5 mg O_3/L was achieved among the replicate experiments for all the ozone doses.

Using reactor A, Smith et al. (1991) found that the amount of ozone which diffused into the effluent was negligible, during the 10 minutes the ozone/oxygen mixture was flowing through the reactor. Therefore, in this study the pre-absorbed ozone was ignored.

The COD reduction from ozone treatment was as expected. Ozone oxidizes chemical bonds, which results in less dichromate needed to completely oxidize the sample, hence lowering COD values. The TOC reductions ranged from 10.5 mg/L (4.3%) for 84.8 mg O_3/L ozone dose to 17.6 (6.4%) for 192.4 mg O_3/L ozone dose. These results are very similar to those found by Mohammed and Smith (1991). In their study the TOC reductions were 10 to 20 mg/L for 50 to 200 mg/L ozone doses of a secondary effluent from a pulp mill. It is possible that some of the smaller molecular weight organics, formed during ozonation, are purged out of the wastewater with the flow of ozone/oxygen gas. This would result in a small loss of TOC and COD in the resulting ozonated wastewater. In reactor A, these volatile organics could be present in the gas phase during the mixing process, and then purged out into the KI traps. In reactor B, the volatiles would be directly purged out of the reactor into the KI traps.

The lower reductions of TOC compared to COD for equivalent ozone doses indicates very little complete oxidation of the organics to carbon dioxide and water. Chemical bond or side chain oxidation would appear to be more prevalent at the ozone doses studied.

5.4.1. Molecular Weight Distributions

Tables 12 and 13, and 15 to 17 detail the molecular weight distribution area characterization data for the effluents and the ozonated effluents. In looking at these tables and figures 12 and 13 there appears to be a reduction of organic material as the effluents were ozonated. The decrease in distribution area was larger as the ozone dose increased, with the percentage decreases being 15.3, 25.5, and 37.1 for respective ozone doses of 84.8, 111.6, and 192.4 mg O_3/L. This decreases in distribution areas are illustrated in figure 19. However, this loss in distribution area does not correlate to the corresponding loss in total organic carbon. The reductions in TOC ranged from 4.3% for 84.8 mg O_3/L ozone dose to 6.4% for 194.6 mg O_3/L ozone dose. The loss in distribution area is therefore due more to a lack of detection of the ozonated organic molecules than an actual loss or complete oxidation of certain organic molecules.

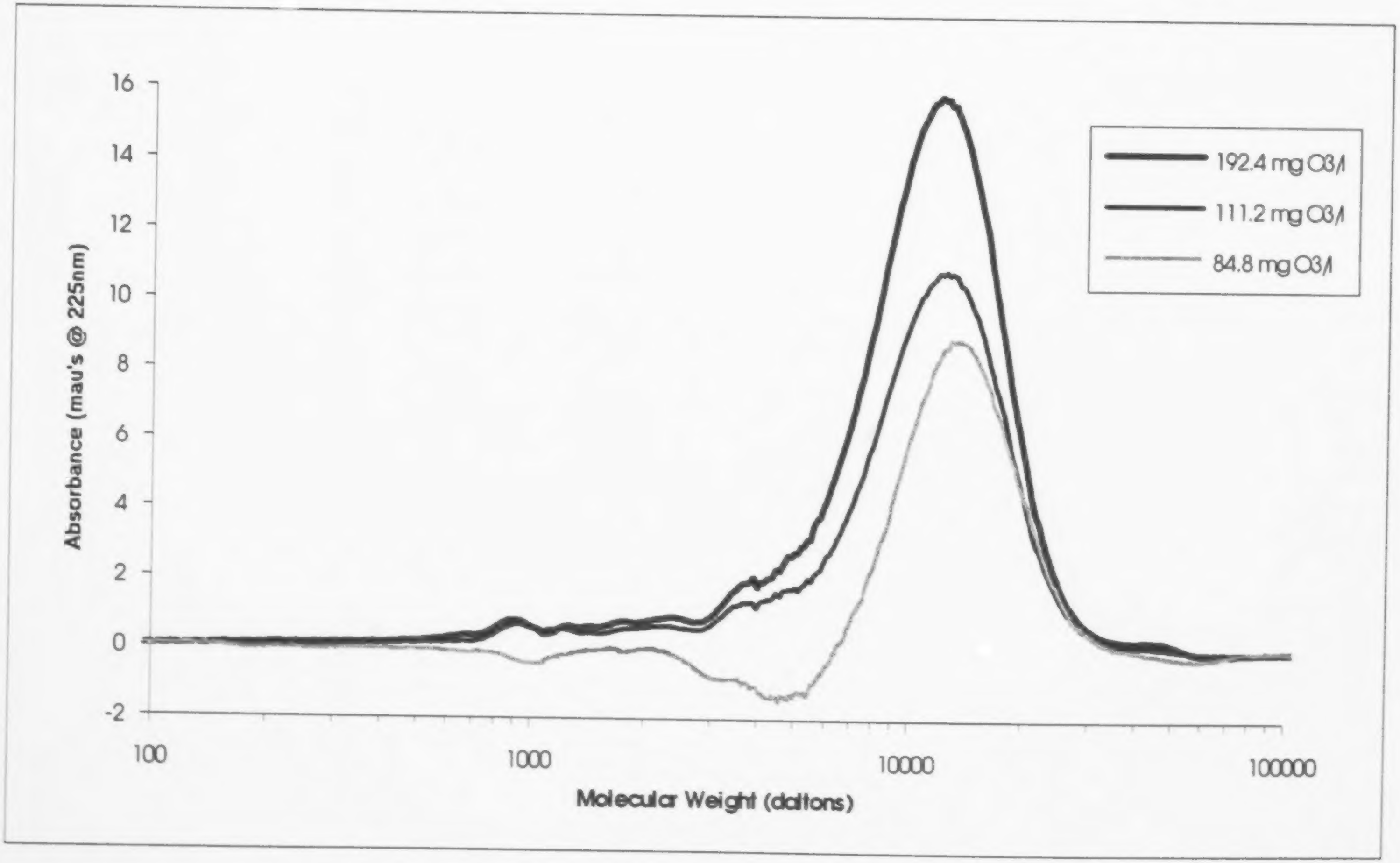

Figure 19: MWD's of Sample Lost during Ozonation (ie., not detected at 225 nm)

The relationship between ozone doses and the decreases in distribution areas are shown in figure 20. As shown by the high R^2 value (0.98) this relationship is linear for the ozone doses studied. This relationship could have important consequences to the measurement of ozone doses in the industrial scale ozonation of pulp mill wastewaters. If this relationship was found to be true for a range of ozone doses on different pulp wastewaters, then the SEC molecular weight distribution area could be used as a tool to approximate the amount of ozone, which reacted with the wastewater.

This lack of detection of the ozonated organics at a wavelength of 225 nm is due to a lower absorptivity which is the result of the oxidation of the bonds in these molecules. Two mechanisms by which ozone oxidizes organic molecules are cyclo addition and electrophilic reaction (Bailey, 1978). In the cyclo addition mechanism the ozone molecule leads to a 1-3 dipolar cyclo addition on unsaturated bonds. The general result of this cyclo addition is a breakdown of the double bond giving two carbonyl compounds and a hydrogen peroxide molecule. The electrophilic reaction is restricted to molecular sites with a strong electronic density such as aromatic compounds (Bailey, 1978). The result of this reaction is the addition of a hydroxyl group onto the aromatic ring. These hydroxylated compounds are susceptible to further ozonation which can lead to the formation of quinoid products. Even further ozonation can produce aliphatic compounds with carbonyl and carboxyl functions (Bailey, 1978). These types of compounds would again have limited absorbency above 200 nm whereas the original aromatic compounds absorb strongly in the near UV range (Parikh, 1974). Also, the loss of the carbon double bonds due to cyclo addition leads to a further loss of absorptivity at the 225 nm wavelength.

Bonnet et al. (1989) studied the by-products from ozonation of a commercial lignin, using gas chromatography - mass spectrometry analysis. Their results showed that ozone doses of less than 0.8 mg O_3 consumed per mg of dissolved organic carbon (DOC) decreased the aromaticity of the lignin and lead to the production of carboxylic acids. For ozone doses of <0.5 mg O_3 consumed / mg DOC many aromatic acids were identified and for ozone doses of >0.5 mg O_3 consumed / mg DOC aliphatic acids were identified as the major oxidation by-products. This indicates that there is significant aromatic ring oxidation in lignin molecules at higher ozone doses. In our study the ozone dosage per TOC was 0.3, 0.4 and 0.7 mg O_3 consumed / mg TOC for the three ozone runs. If we assume the TOC of the suspended solids is very low then the TOC of the ASB effluent samples can be assumed to be very close to the DOC. This indicates that for the highest ozone dose of 192.4 mg O_3/L there may be significant aromatic ring oxidation, while there could be some aromatic ring oxidation for the lower ozone doses. This further explains the loss of ultraviolet absorptivity and color in the ozonated samples.

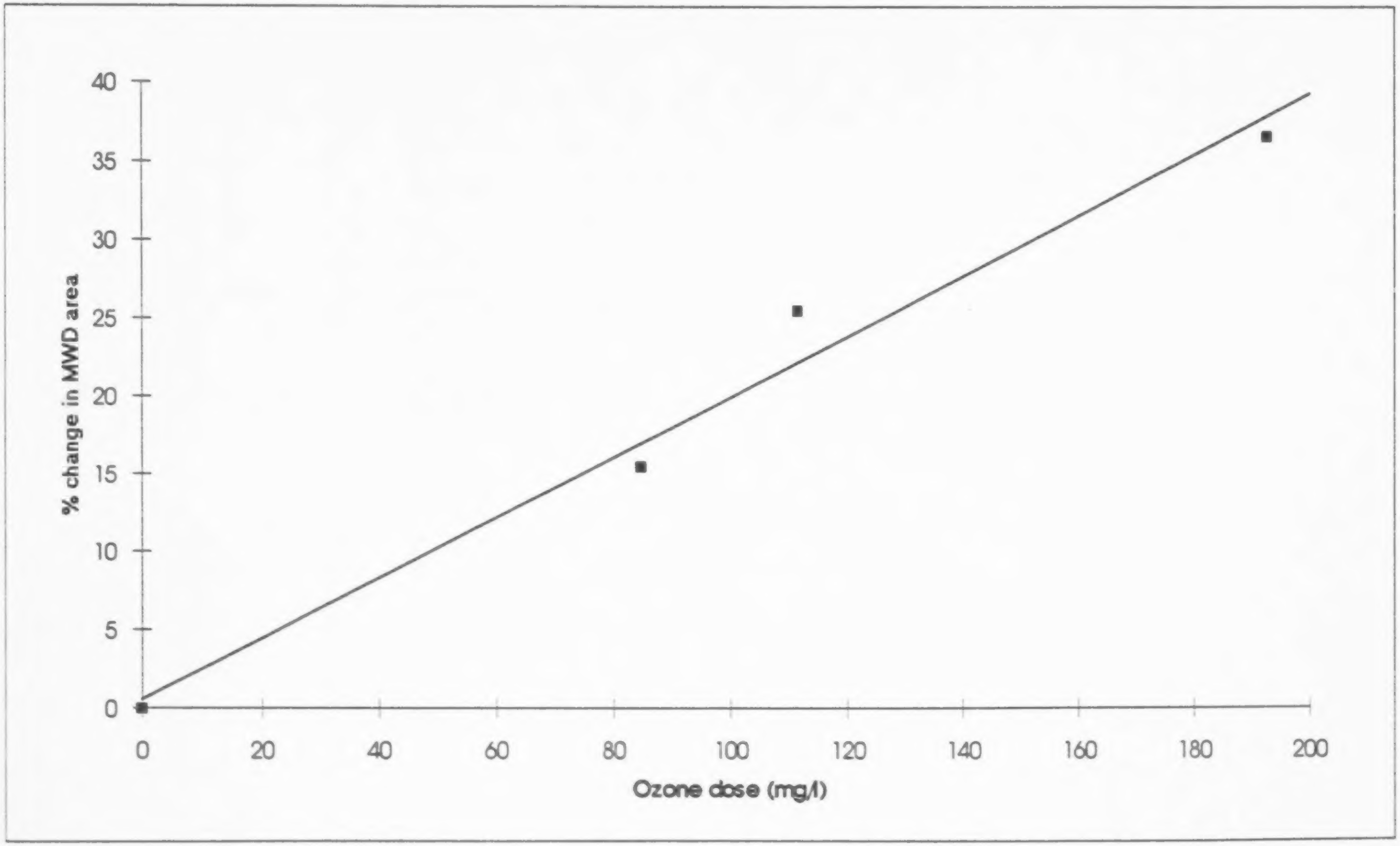

Figure 20: %Change in Molecular Weight Distribution Area vs Ozone Dose ($R^2 = 0.98$)

Roy-Arcand et al. (1991) achieved similar results to our study in terms of the reduction of molecular weight distribution area. After a 250 mg O_3/L application to a kraft bleaching effluent, the distribution area was reduced by 37%. In the Mannson and Oster (1988) study the applications of 1.2 and 3.5 g O_3/g lignin to a kraft lignin sample showed decreasing molecular weight distribution areas, with the 3.5 g O_3/g lignin dose showing a substantial decrease. Area numbers were not listed for the molecular weight distributions in this study. In the Stern and Gasner (1974) study, an application of 1.5 O_3/g lignin to a commercial lignin gave a distribution area reduction of 50%. However, this reduction can be assumed to be equivalent to organic carbon reduction because the detector used was a refractometer.

As shown in tables 12 and 13, and 15 to 17 the weighted average molecular weights were lower for the ozonated effluents. However, there appears to be no trend as the WAMW reductions are 12.6, 7.2 and 12.4% for respective ozone doses of 84.8, 111.6 and 192.4 mg O_3/L. One would expect that the WAMW would decrease in relation to the increase in ozone so that the higher the ozone dose the lower the WAMW. One reason for the apparent random WAMW reductions could be due to two different starting effluents. For, the expected trend is observed with effluent A as the WAMW reductions are 7.2 and 12.4% for respective ozone doses of 111.6 and 192.4 mg O_3/L.

In the Roy-Arcand et al. (1991) study visual inspection of the molecular weight distributions indicate that the WAMW did not change with an ozone dose of 250 mg/L of a kraft bleaching effluent. In the Mannson and Oster (1988) paper on ozone dose of 1.2 g O_3/L lignin did not appear to change the WAMW of the molecular weight distribution. However, an ozone dose of 3.5 g O_3/g lignin did noticeably lower the WAMW. In the Stern and Gasner (1974) study the WAMW decreased with an ozone application of 0.03 g O_3/g lignin and decreased further with an ozone application of 1.5 g O_3/g. In all three studies the change in WAMW had to be determined by visual inspection of the molecular weight distributions, due to the absence of numerical data.

The F1 ultrafiltration fractions of effluent B and ozonated effluent B (84.8 mg O_3/L) were equal in terms of their molecular weight distribution data, as shown in tables 14 and 18. The ozonated F2 MWD had slight decrease in distribution area and a slight increase in WAMW, compared to the original F2, as shown in tables 14 and 19. The decreases of molecular weight distribution area upon ozonation were 13.9% and 31.2% for fractions F3 and F4 respectively (Tables 14, 20 and 21). In both fractions the WAMW and the upper range both decreased with ozonation. The increasing decrease in MWD area as the molecular weight fraction increases during ozonation is due to a higher reactivity of larger lignin molecules to oxidation by ozone. A higher abundance of unsaturated bonds in compounds with higher molecular weights leads to this higher reactivity.

5.4.2. Comparisons of Molecular Weight Distribution with other Analyses

5.4.2.1 Color

As already discussed the reduction in molecular weight distribution area ranged from 15.3% for an ozone dose of 84.8 mg O_3/L to 37.1% for an ozone dose of 192.4 mg O_3/L. Color reduction data is not available at this time so for comparative purpose the Mohammed and Smith (1991) color reductions will be looked at. The color reductions in this study for secondary effluent were 60, 82.5, 89.8 and 87.4% for respective ozone doses of 50, 100, 150 and 200 mg O_3/L. The oxidation of unsaturated bonds and aromatic bonds, have a more pronounced effect on the visible detection region than the ultraviolet detection region. The color causing chemical bonds in organic molecules are comprised of unsaturated bonds such as C=C and N=N (Parikh, 1974). When these bonds are oxidized through ozonation the color is reduced. It is apparent that the oxidation of these bonds reduces the color to a much greater extent than the ultraviolet absorbance.

5.4.2.2. Total Organic Carbon

As shown in Appendix C, the 225 nm wavelength was chosen due to equivalent distribution area over TOC ratios for each ultrafiltration fraction.

However, the change in distribution area of the MWDs of the ozonated effluent ultrafiltration fractions compared to the original fractions do not correlate very well to the corresponding change in total organic carbon. For fractions F4, F3 and F2 the distribution area decreases and the TOC values decrease upon ozonation. The distribution area decreases 29.7%, 13.9% and 20.7% while the TOC decreases 19.3, 12.6 and 6.9 for fractions F4, F3, and F2 respectively. However, for fraction F1 there is no change in distribution area while the TOC increases 35.4%, upon ozonation. This is an indication that there is a large amount of organic material in this low molecular weight fraction of ozonated wastewater that is not detected in the UV range. As discussed before, this is probably due to a lack of UV absorbing bonds (i.e., C=C) in the lower molecular weight range.

5.4.2.3. Chemical Oxygen Demand

As in the TOC results there is a corresponding COD reduction to the MWD area reduction in ultrafiltered fractions F4, F3, and F2 of ozonated effluent. The COD reductions were 24.1%, 10.9% and 4.5% for F4, F3, and F2, respectively. However, just as in the TOC the F1 fraction showed an increase (25.5%) in COD upon ozonation. This is further evidence that some organic compounds in this fraction are not "seen" by the UV-detector.

6. CONCLUSIONS

- The molecular weight distribution calibration data indicated that the molecular size exclusion results correlated very well to molecular weight exclusion results under the chromatographic conditions used.

- BOD_5 reduction from the primary to secondary effluent was 95% which showed that the aerated stabilization basin was operating very efficiently.

- The reduction in molecular weight distribution area was much lower than the reduction in total organic carbon from the primary to secondary effluent. This indicated a lack of detection of lower molecular weight compounds with the UV-detector.

- The color increased with increasing molecular weight as shown by analysis of the secondary effluent ultrafiltration fractions. A straight line relationship was found between the color/TOC ratio and the weighted average molecular weights of the secondary effluent ultrafiltration fractions.

- Molecular weight distributions of the ultrafiltration fractions of ASB effluent showed a lack of correlation to the manufacturer's cut-off numbers. This was not unexpected as the actual rejection coefficient of the membranes is sample specific.

- A straight line relationship was found between the change in molecular weight distribution areas of ozonated secondary effluents and the ozone dose applied.

- A comparison of TOC and MWD results indicated that there were lower molecular weight compounds in the ozonated effluent that were not detected by the UV detector. COD results further confirmed this observation. This was due to lower UV absorptivities of chemical bonds oxidized by ozone.

- For effluent A the weighted average molecular weight decreased 7.2 and 12.4% for ozone doses of 111.6 and 192.4 mg O_3/L. These results were as expected according to the literature. However, the reduction of the WAMW for effluent B at an ozone dose of 84.8 mg O_3/L was 12.6%, which indicated that the percentage change in WAMW per ozone dose may be sensitive to a slight change in starting material.

- The higher the molecular weight ultrafiltration fraction, the higher the decrease in distribution area upon ozonation. This was due to a higher reactivity towards ozone for larger molecules, which have an abundance of unsaturated bonds.

7. RECOMMENDATIONS

- Use a detection device, for size exclusion studies of pulp mill wastewater ozonation, that would more accurately quantify the amount of organics. A TOC or mass-spec detector would be worth pursuing.

- Use higher ozone doses to further study the degradation of ASB effluent molecules using molecular weight distributions.

- Ultrafilter polyethylene glycol standards and then study the size exclusion results of these fractions in order to see if the membrane cut-offs are more accurate for these calibration standards.

-Use Analytical tools such as ^{1}H-NMR and mass spectrometry in order to determine the extent of aromatic ring oxidation when ozonating pulp mill wastewaters.

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APPENDIX A

Chemical Oxygen Demand Method

1. An approximately 0.25M ferric ammonium sulfate (FAS) solution was made. This solution was the secondary standard and was standardized using the primary standard of 0.250 N potassium dichromate ($K_2Cr_2O_7$).

2. Approximately 1 g of mercuric sulfate ($HgSO_4$) was added to a reflux flask.

3. 20 mL of sample was added using volumetric pipettes, in duplicate. For the ultrafiltration fractions 50 mL was added. Also, for the two blanks 50 mL of Milli-Q grade water was added.

4. Milli-Q grade water was added, using a graduated cylinder, to make-up the volume to 50 mL.

5. 10 mL of Ag_2SO_4/H_2SO_4 solution was added, using a graduated cylinder in order to dissolve the $HgSO_4$. This solution was made by adding 22.7 grams of reagent grade silver sulfate to a full 2.25 liter bottle of concentrated sulfuric acid

6. 20 mL of 0.250 M potassium dichromate ($K_2Cr_2O_7$) solution was added with a volumetric pipette.

7. 45 mL of Ag_2SO_4/H_2SO_4 solution was added, using a graduated cylinder.

8. A few glass beads were added.

9. Refluxed for 2 hours, and then solution was cooled to room temperature.

10. For the duplicate primary standard solutions 50 mL of Milli-Q grade water was added and then steps 4 through 6 were followed.

11. The duplicate primary standard solutions were titrated with the FAS solution, using Ferroin as an indicator, to a brick red endpoint. The concentration of the FAS solution (in eq/L) was then obtained using the following formula:

    $$[\text{FAS}](\text{eq/L}) = \{(20.00\ \text{mL}) \times (0.250\ \text{eq/L})\} + (\text{titrant volume in mL})$$

12. The refluxed solutions are then titrated with the standardized ferric ammonium sulfate (FAS) solution using Ferroin as an indicator. The COD values for each sample were then obtained using the following formula:

$$\text{COD (mg/L)} = 8000 \times \frac{[(\text{blank titrant vol (mL)} - \text{sample titrant vol (mL)})] \times [\text{FAS}](\text{eq/L})}{(\text{sample vol (mL)})}$$

Ultrafiltration Method

This method was as outlined by H. Mao (personal communication, 1994)

1. The sample was filtered first through a Whatman glass-fiber filter (934AH) and then through a 0.8 µm membrane filter (MSI Micron Separations Inc.) in order to remove the suspended solids.

2. The 10,000 MWCO membrane was set up in the system.

3. The pressure was set to 15 psi using the pump .

4. 2.0 liters of the sample was used and the sample container was put in an ice bath to help preserve it.

5. The sample was concentrated down to approximately 5% of the original volume (50 L).

6. The retentate was then washed three times with a total of approximately 1 liter of milli-Q water.

7. The washing water was then collected.

8. The 5000 and the 1000 MWCO were then used, respectively, in the system following steps 5 through 7 for each run.

9. The wash water was then collected at the end and used in calculations of the < 1000 fraction.

Each membrane was cleaned by pumping 200 mL of 0.1N NaOH through it for 30 minutes, followed by pumping 2 L of mill-Q water through it for 30 minutes. The membranes were then stored in the buffered solution recommended by the manufacturer.

APPENDIX B

SEC System User's Manual

Appropriate Samples:	Bleach Effluent Primary Effluent Secondary Effluent Ozonated Effluents Biologically Treated Effluents
Column:	Ultrahydrogel 250 (7.8 mm id x 30 cm) with Ultrahydrogel Guard Column
Mobile Phase:	25 mM Carbonate Buffer (Na_2CO_3 / $NaHCO_3$) pH = 10.3
Molecular Weight Stds:	Peptide Mixture - mol wts. = 814, 1569, 3079, 6158 Polyethylene Glycol Mixture 194, 600, 4100, 12600 Pullulan Mixture 5800, 12200, 23700, 48000
Detection Wavelengths:	225 nm for Samples 225 nm for Peptide Standards 245 nm for PEG and Pullulan Standards

SOLUTION PREPARATIONS:

Mobile Phase:

1) Add 2.650 grams Na_2CO_3 and 2.100 grams $NaHCO_3$ (weighed accurately) to a 2 liter volumetric flask and dilute to the mark with milli-Q H_2O

2) Filter through a 0.45 µm HVLP filter (Millipore)

Samples:

1) First make-up 50 mM Carbonate Buffer:
   Add 2.650 grams Na_2CO_3 and 2.100 grams $NaHCO_3$ (weighed accurately)
   to a 1 liter volumetric flask and dilute to the mark with milli-Q H_2O
   Filter through a 0.45 µm HVLP filter (Millipore)

2) Filter samples through a 0.45 µm HVLP filter (Millipore)

3) Dilute Samples 1:1 with 50 mM carbonate buffer solution (i.e., 1000 µL wastewater / 1000 µL 50mM carbonate buffer)

Standards:

Pullulans - Add 0.02 g (20 mg) and 1.000 mL of 25 mM carbonate buffer for P-5, P-10, P-20 and P-50 to make ~2% solutions.
Run each Pullulan separately.

Polyethylene Glycols (PEG)

Add 20 mg and 1.000 mL of 25 mM carbonate buffer for PEG 600, 4100 and 12600 to make ~2% solutions. Add 30 mg and 1.000 mL of 25 mM carbonate buffer for PEG 194 to make ~3% solution.
Run these samples as a mixture by adding equal volumes together.
(i.e., 500 µL of each into a separate vial)

Peptides Mixture

Already made-up in milli-Q H_2O.
Dilute 1:1 with 50 mM carbonate buffer solution
(i.e., 1000 µL peptide mixture / 1000 µL 50mM carbonate buffer)

HPLC SYSTEM SET-UP:

1) Degas the mobile phase, and set up the HPLC system according to the HPLC operation manual written by the Environmental Health department of the U of A. Follow only points 1 through 6 of the start-up procedure, as points 7 through 9 are for reverse phase columns.

2) Set the high pressure limit on the pump to 1500 psi or 150 on the readout, as the maximum pressure is 10x the LED readout.

3) Flow the mobile phase through the column for at least one hour before starting the run.

4) Continue on to the computer software set-up on the next page (HPCHEM Set-up).

HPCHEM SYSTEM SET-UP

View Menu:

Select *Method and Run Control*

Instrument Menu:

Select *Control* from *More DAD*

Select *On* in *Lamp* Section

Select *Normal* in *Lamp Current* Section

Method Menu:

Method to be used - SECDAD

Select *Edit Entire Method*

Check off all method sections to edit and select *OK*

Method Comments

Size Exclusion on Ultrahydrogel Column (250)
Select *OK*

DAD Signals

Set the signals as follows

	Sample, BW		*Reference, BW*	
A:	*225*	*4*	*450*	*80*
B:	*245*	*4*	*450*	*80*
C:	*208*	*4*	*450*	*80*
D:	*215*	*4*	*450*	*80*
E:	*235*	*4*	*450*	*80*
F:	*254*	*4*	*450*	*80*

Peak Width	*Stop Time*	*Auto balance*
0.053	*20.00*	*ON*

Method Menu (cont'd):

Signal Details

Confirm that the information in the table is equivalent to that in the DAD Signals Section, along with the 20.00 stop time. Select *OK*

Integration Events
Select *OK* (can be changed when printing out chromatograms after the run)

Specify Report
Select *Screen* in *Destination* Section
(Reports should be printed out later when the chromatograms are integrated properly, and the absorbance scales are set properly.)

Sequence Recalibration Table
Select *OK*
(This type of calibration is used for quantitative HPLC analysis such as reverse phase HPLC)

Run Time Checklist : HPLC System
Check *Data Acquisition* and Standard Data Analysis
Select *OK*

Sequence Menu:

Select *Sequence Parameters*
Enter *Operator Name*
Enter MonthDay into *Subdirectory* (i.e. *APR7*)
Note: It is very important that this subdirectory be changed for each subsequent run, or previous run data could be over written.

Select *Sample Table*
Enter *Sample Name*; *Sample Amount* (Volume injected); multiplier = *1*
Enter *Sample Info*: Stds - *w/v %*; Samples - *dil 1:1 50 mM* Na_2CO_3 / $NaHCO_3$

Sequence Menu (cont'd):

Select *Sequence Table*

Seq Line	*Method Name*	*From Vial*	*To Vial*	*Inj/Vial*
1	*SECDAD*	*1*	*Last Vial #*	*1*

Press *OK*

Select *Sample Log Table*
Inspect this table to insure that it shows all of the samples to be run
Press *OK*

Select *Print Sequence* and print the *Sample Log Table* to have a hard copy of sample information for each data file.

Autosampler Set-up:

1) Select *Auto* on the *Auto/Single* button

2) Press *Sample no.*

3) Press *0* *Enter*

4) Press *inj vol* *10* *Enter*

5) Press *run time* *20* *Enter*

6) Press *equil delay* *5* *Enter*

7) Press *Enter*

If the *inj vol* has to be changed for certain sample numbers press *Sample no.* button until the desired sample number is reached and enter the appropriate inj vol and press *Enter* twice.

8) Load sample vials on the tray in the appropriate positions

Run Control Menu:

Select *Run Sequence*

Press Start Run on the autosampler and the run is underway.

SHUT-DOWN PROCEDURE:

1) Flush the system with milli-Q water (0.45 μm filtered and degassed) for at least one hour.

2) Stop flow from the pump and enter 0.0 mL/min for the flow rate and 0% for all the solvents.

3) Press the standby button and then the idle button on the DAD detector. If the singular wavelength UV-Vis detector is being used turn off the ADC interface and then the detector.

4) Turn off the WISP autosampler.

POST RUN DATA ANALYSIS:

View Menu:

Select *Data Analysis*

File Menu:

Select *Load Signal*
Select File and Wavelength(s)
Check *File Info* to ensure the file selected is the correct one
Press *OK*

Integration Menu:

Select *Integration Events* and set the initial values as follows:
Initial Area Reject = 0
Initial Peak Width = 0.400
Initial Threshold = -3.000
Press *OK*

Select *Integrate* and check to see that the chromatogram is integrated properly. If not, change the integration events slightly until the peaks are integrated properly.

POST RUN DATA ANALYSIS (cont'd):

Report Menu:

Select *Customize Report*

Select *Layout Designer*

Load Template sec3.tpr and edit the mobile phase, flow and pressure fields, if required (consult the HPCHEM user's manuals).

Select *Specify Report*

Select Printer in *Destination* Section
Select *SEC1* in *Style* Section

Select *Print Report*

When done printing out the reports exit the HPCHEM software and shut off the computer.

Ordering information:

Their are two suppliers for all of the products required as follows:

Millipore Corporation
Waters Chromatography Division
3688 Nashua Drive
Missisauga, Ontario
L4V 1M5
Phone # 1-800-268-4881

Fisher Scientific Co.
10720 - 178th Street
Edmonton, Alberta
T5S 1J3
Phone # 483-2123

Product	**Supplier**	**Catalogue #**
Ultrahydrogel 250 Column	Millipore	11525
Ultrahydrogel Guard Column	Millipore	11565
0.45 µm Membrane filters	Millipore	HVLP 04700
Pullulan Standards Kit, P-82	Millipore	34207
Polyethylene Glycol Stds. Kit	Millipore	35711
4 mL Autosampler vials (144)	Fisher	03-340-5M
Caps for autosampler vials (144)	Fisher	03-340-7C
Septa for autosampler vials (144)	Fisher	03-340-11C
Inserts for autosampler vials (144)	Fisher	03-340-17G
Na_2CO_3 (500g reagent grade)	Fisher	S263-500
$NaHCO_3$ (500g reagent grade)	Fisher	S233-500

APPENDIX C

Mobile Phase Selection

The testing of various mobile phases is the part of the SEC method development which required the most amount of time.

The mobile phases tested are listed in table C.1 along with a rating of the various performance characteristics. The ratings are as follows:

A - Excellent

B - Very good

C - Good

D - Fair

E - Poor

For the sample adsorption section a rating of A indicates that the sample does not have adsorption problems and a rating of F indicates the sample has vast adsorption problems using the indicated mobile phase. In general, for the calibration ratings an r^2 value between 0.93 and 1.0 gives a rating of A, an r^2 value between 0.86 and 0.93 gives a rating of B and an r^2 value between 0.79 and 0.86 gives a rating of C. Further explanation of the table 3 ratings along with other mobile phase characteristics are detailed in the paragraphs on the pages following table 3.

Table C.1: Performance ratings for the various mobility phases tested during SEC work.

Mobile Phase	pH	Calibration Linearity				Correlation to UF Fractions	Sample Adsorption	Other Problems
		PEG	Pull	Pept.	All			
10 mM Tris-HCl	8.0	A	A	-	B	D	C	
50 mM P/0.1 M NaCl	6.8	C	-	-	-	C	A	
50 mM P/0.1 M KCl /6 M Gu	7.0	C	-	-	-	D	A	Proper rinsing of HPLC is crucial to remove Guanidine
20 mM Anm AC	6.5	A	A	A	C	F	F	
50% H_2O/50% MeOH	n/a	A	-	-	-	F	A	
50% 20 mM Amm AC 50% MeOH	n/a	A	C	A	B	B	A	Amm Ac precipitated out (% MeOH too high)
70% 20 mM Amm Ac 30% MeOH	n/a	A	A	A	A	F	D	
0.01 m NaOH	11.3	C	A	A	C	D	A	
50 mM P	6.8	A	A	A	A	C	A	Correlation with TOC of UF fractions not good
25 mM $NaHCO_3$	10.3	A	A	A	A	B	A	
25 mM $NaHCO_3$ 0.5 g/l PEG 6000	10.3	A	A	A	A	B	A	Foaming problem when degassing

Tris = tris(hydroxymethyl)aminomethane P = phosphate Gu = Guanidine- HCl Amm Ac = Ammonium acetate MeOH = Methanol

The 10 mM Tris-HCl mobile phase had r^2 values of 0.993, 0.999 and 0.89 for the PEG, Pullulan and combined standards which resulted in ratings of A, A and B, respectively. The peak molecular weights for the ultrafiltration fractions F4, F3, F2 and F1 were 11157, 10500, 10206 and 9336, respectively. This led to a correlation rating of D, largely due to the low resolution of the fractions. For this mobile phase, the chromatogram area was much lower than expected when larger volumes of undiluted wastewater were injected (15 μL) when compared to injections of diluted wastewater. This indicates a problem with sample adsorption at higher column loadings. However, this adsorption problem did not occur at lower injection volumes (5 μL) or upon injection of diluted wastewater (1:3 or 1:5), therefore a rating of B was given for sample adsorption.

For the 50 mM phosphate/0.1 M NaCl mobile phase the r^2 value was 0.98 for the PEG standards. However, there was very high spreading of the PEG peaks which led to poor resolution, probably due to the high ionic strength. Therefore, the rating was lowered to C due to this poor resolution. The peak molecular weights for the ultrafiltration fractions F4, F3, F2 and F1 were 6092, 4797, 4347 and 3763, respectively. This led to a correlation rating of C, largely due to the low resolution of the fractions. There did not appear to be any adsorption problems with this mobile phase and so a rating of A was given.

The 50 mM phosphate/0.1 M KCl/6M guanidine-HCl was tested in order to see if there were problems with association between the wastewater molecules. Guanidine-HCl is a denaturing agent and would therefore alleviate association effects. The chromatograms obtained with this buffer were almost identical to those obtained with the 50 mM phosphate/0.1 NaCl. Therefore, it can be assumed that association complexes are not formed within the pulp mill wastewaters looked at. This high concentration of guanidine in this mobile phase caused a concern with salting-out effects, therefore thorough rinsing of the HPLC system was very important. There did not appear to be any adsorption problems with this mobile phase and so a rating of A was given.

The 20 mM ammonium acetate mobile phase had r^2 values of 0.98, 0.99, 0.98 and 0.80 for the PEG, Pullulan, Peptide and combined standards, which resulted in ratings of A, A, A and C, respectively. The peak molecular weights for the ultrafiltration fractions F4, F3, F2 and F1 were 9613, 12209, 12705 and 7518, respectively. Therefore, a rating of F was given for fraction correlation. There was a huge problem with sample adsorption using this mobile phase. There was an increase in the distribution area and the distributions became narrower, with subsequent wastewater runs, even at low column loadings. Therefore, a rating of F was given for adsorption. The fraction correlation problems were partially due to this adsorption problem.

For the 50% H_2O / 50% MeOH only two PEG standards were run (PEG-12600 and PEG-4100), so the r^2 value was obviously 1.000. Only two standards were run in order to check the location of the wastewater peaks. Using the ASB effluent runs it was estimated that the F1 fraction would have a peak molecular weight of >24,000, which leads to a rating of F for fraction correlation. There were no sample adsorption problems and so a rating of A was given. In fact, after trying other cleaning agents, this mobile phase was found to be very good in cleaning out the column of adsorbed wastewater.

The 50% 20 mM ammonium acetate / 50% MeOH mobile phase had r^2 values of 0.97, 0.86, 0.96 and 0.88 for the PEG, Pullulan, Peptide and combined standards, which resulted in ratings of A, C, A and B, respectively. The peak molecular weights for the ultrafiltration fractions F4 and F1 were estimated from the ASB effluent chromatogram at 23000 and 3000, respectively. Therefore, a rating of B was given for the fraction correlation. There were pumping problems due to precipitation of the ammonium acetate in the pump filter. Very unstable baselines also indicated a salting-out effect in the buffer. The precipitation problems were probably due to the high concentration of MeOH. Therefore, the MeOH was reduced to 30% for the next mobile phase. There did not appear to be any adsorption problems with this mobile phase and so a rating of A was given.

The 70% 20 mM ammonium acetate / 30% MeOH mobile phase had r^2 values of 0.997, 0.995, 0.997 and 0.96 for the PEG, Pullulan, Peptide and combined standards, which resulted in ratings of A, A, A and A, respectively. The peak molecular weights for the ultrafiltration fractions F4, F3, F2 and F1 were 28916, 18676, 15223 and 7976, respectively. Therefore, a rating of F was given for fraction correlation. With subsequent ASB effluent runs the distribution area increased by as much as 21%, which is an indication of a sample adsorption problem. Therefore, a rating of D was given for adsorption.

The 0.01M NaOH mobile phase had r^2 values of 0.99, 0.99, 0.96 and 0.80 for the PEG, Pullulan, Peptide and combined standards, which resulted in ratings of C, A, A and C, respectively. There was very high spreading of the PEG peaks which led to poor resolution, probably due to the high pH. Therefore, the rating for this set of standards was lowered to C. The peak molecular weights for the ultrafiltration fractions F4, F3, F2 and F1 were 10089, 8152, 7133 and 6104, respectively. Therefore, a rating of D was given for fraction correlation. There did not appear to be any adsorption problems with this mobile phase and so a rating of A was given.

The 50 mM phosphate mobile phase had r^2 values of 0.99, 0.97, 0.99 and 0.97 for the PEG, Pullulan, Peptide and combined standards, which resulted in ratings of A, A, A and A, respectively. The peak molecular weights for the ultrafiltration fractions F4, F3, F2 and F1 were 7578, 5372, 4252 and 3098, respectively. The F3, F2 and F1 fractions had distribution area / TOC ratios, which were too low compared to the ratio for F4 at a wavelength of 208 nm. Therefore, a rating of C was given for fraction correlation. There did not appear to be any adsorption problems with this mobile phase and so a rating of A was given.

The 25 mM $NaHCO_3$ mobile phase had r^2 values of 0.99, 1.000, 0.99 and 0.93 for the PEG, Pullulan, Peptide and combined standards, which resulted in ratings of A, A, A and A, respectively. The weighted average molecular weights for the ultrafiltration fractions F4, F3, F2 and F1 were 11551, 7458, 5777 and 3663, respectively (see table 16).

Therefore, a rating of B was given for fraction correlation. As show in table C.2 of this Appendix the area/TOC ratios at a wavelength of 225 nm were very good. There did not appear to be any adsorption problems with this mobile phase and so a rating of A was given. This was the mobile phase chosen for the molecular weight distribution analyses, as overall it had the best performance.

The PEG 6000 was added to the 25 mM $NaHCO_3$ buffer to see if there were any problems with the sample adsorbing to the column due to hydrogen bonding. Pellinen and Salkinoja-Salonen (1985b) suggested PEG 600 would saturate the adsorption sites on the column by forming hydrogen bonding thereby alleviating sample adsorption to these sites. The Chromatograms using the carbonate buffer were identical with and without the PEG 6000. Therefore, hydrogen bonding does not appear to be a problem with this buffer. This mobile phase was not used due to a large problem with foaming during the degassing procedure.

Detection Wavelength Selection

UV - Vis spectrums of the primary effluent, secondary effluent and ultrafiltration fractions were obtained. 208 nm was chosen as the initial wavelength for the SEC studies, based on these spectra.

With the use of the diode array detector several (7) wavelengths could be used to monitor the column eluent during one run. The most suitable wavelength should be that which gives quantification of the ultrafiltration fraction, which correlate to the amount of organic carbon in the sample. The results of the area per TOC ratios for the suitable wavelength should be consistent. The wavelength of 225 nm was found to have the best consistency in terms of the area/TOC ratios, as shown in Table C.2. Higher wavelengths showed decreasing area/TOC ratios for the lower fractions of F2 and F1, and therefore were not suitable.

For the peptide standards 225 nm was found to be good and for the PEG and Pullulan standards 245 nm gave the best resolution and peak shape.

Table C.2: Area/TOC ratios for UF fractions at different wavelengths

ASB Eff Fraction	*Area (208 nm)*	*Area (215 nm)*	*Area (225 nm)*	*TOC (mg/L)*	$\frac{Area(208)}{TOC}$	$\frac{Area(215)}{TOC}$	$\frac{Area(225)}{TOC}$
F4	2147.3	1669.4	1334.6	113.4	18.94	14.72	11.77
F3	870.0	653.2	502.7	46.3	18.79	14.11	10.86
F2	864.2	666.6	396.0	35.8	24.14	18.62	11.06
F1	1746.4	1198.0	529.8	52.1	33.52	22.99	10.17

Vapor Pressure Osmometry

In order to achieve a more accurate molecular weight calibration VPO analysis of the ultrafiltration fractions was attempted. The F4 (>10,000 Da) and F2 (5000-1000 Da) fractions were first freeze dried in order to remove all of the water. The solid samples were then mixed with common VPO solvents to try to dissolve them. The samples were totally insoluble in pyridine, acetone, methylene chloride and THF, and were only sparingly soluble in dichloro(o)benzene.

Therefore, water had to be used for the VPO analysis. Water is not a common VPO solvent due to low thermistor responses with the units using this solvent.

Using sucrose as a standard (MW = 342.3 Da) a VPO analysis was tried on the F4 and F2 fractions. The results were 538.1 Da for F4 and 101.4 Da for F2. These very low results indicate that these may be a problem with the ionic nature of the organic molecules in the sample (carboxylic acids) and a formation of ion pairs with anions and cations, in water.

VPO analysis of some of the polyethylene glycol standards was also done. The results were as follows:

PEG MW (Da)	VPO MW (Da)
1470	1429
4100	2864
7100	4008
12600	5538

The deviation at higher MWs is probably due to the insensitivity of the thermistor when using water as the solvent.

APPENDIX D

Statistical Comparisons of MWD Characterization Data

Tables D.1 to D.9 list the results of the Mann-Whitney rank-sum nonparametric test. The null hypothesis (Ho) is rejected or accepted using tabulated values at a probability of 95% (Conover, 1980).

Table D.1: Statistical Comparisons of MWD Characterization Data for ASB Influent A and ASB Effluent A (Data originally shown in tables 10 & 12)

	ASB Inf A		ASB Eff A		rank-sum	rank-sum	Ho
	mean	std dev	mean	std dev	calc.	@ 95%	
Distribution Area	3155	*14*	2750	*31*	6	7	rejected
Weighted Average Molecular Weight	6201	*551*	8002	*541*	6	7	rejected
Number Average Molecular Weight	1638	*335*	1751	*25*	9	7	accepted
Dispersivity	3.9	*1.1*	4.6	*0.4*	9	7	accepted
MW Range: High	21341	*2020*	24603	*1634*	6	7	rejected
Low	249	*37*	489	*119*	6	7	rejected

Table D.2: Statistical Comparisons of MWD Characterization Data for ASB Influent B and ASB Effluent B (Data originally shown in tables 11 & 13)

	ASB Inf B		ASB Eff B		rank-sum	rank-sum	Ho
	mean	std dev	mean	std dev	calc.	@ 95%	
Distribution Area	3185	*108*	2520	*29*	6	7	rejected
Weighted Average Molecular Weight	6363	*25*	8639	*109*	6	7	rejected
Number Average Molecular Weight	1362	*214*	1494	*109*	8	7	accepted
Dispersivity	4.8	*0.8*	5.8	*0.4*	10	7	accepted
MW Range: High	21633	*400*	24471	*408*	6	7	rejected
Low	268	*24*	614	*187*	6	7	rejected

Table D.3: Statistical Comparisons of MWD Characterization Data for ASB Effluent A and 111.2 mg O_3/L ozonated ASB Effluent A (Data originally shown in tables 12 & 15)

	ASB Eff A		**111.2 mg O_3/L**		**rank-sum**	**rank-sum**	**Ho**
	mean	**std dev**	**mean**	**std dev**	**calc.**	**@ 95%**	
Distribution Area	2750	*31*	2048	*60*	***21***	***24***	**rejected**
Weighted Average Molecular Weight	8002	*541*	7427	*312*	***25***	***24***	**accepted**
Number Average Molecular Weight	1751	*25*	2612	*713*	***6***	***9***	**rejected**
Dispersivity	4.6	*0.4*	3.0	*0.6*	***21***	***24***	**rejected**
MW Range: **High**	24603	*1634*	21876	*460*	***21***	***24***	**rejected**
Low	489	*119*	537	*142*	***14***	***9***	**accepted**

Table D.4: Statistical Comparisons of MWD Characterization Data for ASB Effluent A and 192.4 mg O_3/L ozonated ASB Effluent A (Data originally shown in tables 12 & 16)

	ASB Eff A		**192.4 mg O_3/L**		**rank-sum**	**rank-sum**	**Ho**
	mean	**std dev**	**mean**	**std dev**	**calc.**	**@ 95%**	
Distribution Area	2750	*31*	1731	*64*	***21***	***24***	**rejected**
Weighted Average Molecular Weight	8002	*541*	7009	*121*	***21***	***24***	**rejected**
Number Average Molecular Weight	1751	*25*	1498	*241*	***24***	***24***	**accepted**
Dispersivity	4.6	*0.4*	4.8	*0.7*	***13***	***9***	**accepted**
MW Range: **High**	24603	*1634*	18648	*354*	***21***	***24***	**rejected**
Low	489	*119*	606	*96*	***8***	***9***	**rejected**

Table D.5: Statistical Comparisons of MWD Characterization Data for ASB Effluent B and 84.8 mg O_3/L ozonated ASB Effluent B (Data originally shown in tables 13 & 17)

	ASB Eff B		**84.8 mg O_3/L**		**rank-sum**	**rank-sum**	**Ho**
	mean	**std dev**	**mean**	**std dev**	**calc.**	**@ 95%**	
Distribution Area	2520	*29*	2135	*40*	***21***	***24***	**rejected**
Weighted Average Molecular Weight	8639	*109*	7551	*417*	***21***	***24***	**rejected**
Number Average Molecular Weight	1494	*109*	2778	*905*	***10***	***9***	**accepted**
Dispersivity	5.8	*0.4*	3.0	*1.1*	***22***	***24***	**rejected**
MW Range: High	24471	*408*	22750	*1726*	***21***	***24***	**rejected**
Low	614	*187*	618	*213*	***14***	***9***	**accepted**

Table D.6: Statistical Comparisons of MWD Characterization Data for original Ultrafiltration fraction F1 and Fraction F1 of 84.8 mg O_3/L ozonated ASB Effluent B (Data originally shown in tables 14 & 18)

	Eff F1		Ozonated F1		rank-sum	rank-sum	Ho
	mean	std dev	mean	std dev	calc.	@ 95%	
Distribution Area	530	*6*	513	*23*	***24.5***	***24***	**accepted**
Weighted Average Molecular Weight	3663	*337*	3769	*108*	***16***	***9***	**accepted**
Number Average Molecular Weight	1859	*288*	1651	*1122*	***27***	***24***	**accepted**
Dispersivity	2.0	*0.4*	3.1	*1.5*	***11***	***9***	**accepted**
MW Range: High	11944	*2693*	12376	*6162*	***17***	***9***	**accepted**
Low	322	*234*	312	*197*	***29.5***	***24***	**accepted**

Table D.7: Statistical Comparisons of MWD Characterization Data for original Ultrafiltration fraction F2 and Fraction F2 of 84.8 mg O_3/L ozonated ASB Effluent B (Data originally shown in tables 14 & 19)

	Eff F2		Ozonated F2		rank-sum	rank-sum	Ho
	mean	std dev	mean	std dev	calc.	@ 95%	
Distribution Area	396	*20*	314	*12*	***21***	***24***	**rejected**
Weighted Average Molecular Weight	5777	*67*	6156	*141*	***6***	***9***	**rejected**
Number Average Molecular Weight	1958	*512*	4952	*312*	***6***	***9***	**rejected**
Dispersivity	3.1	*0.9*	1.2	*0.1*	***21***	***24***	**rejected**
MW Range: High	11859	*257*	12188	*486*	***10***	***9***	**accepted**
Low	560	*141*	2231	*361*	***6***	***9***	**rejected**

Table D.8: Statistical Comparisons of MWD Characterization Data for original Ultrafiltration fraction F3 and Fraction F3 of 84.8 mg O_3/L ozonated ASB Effluent B (Data originally shown in tables 14 & 20)

	Eff F3		Ozonated F3		rank-sum	rank-sum	Ho
	mean	std dev	mean	std dev	calc.	@ 95%	
Distribution Area	503	*7*	433	*19*	***21***	***24***	**rejected**
Weighted Average Molecular Weight	7458	*99*	7186	*101*	***21***	***24***	**rejected**
Number Average Molecular Weight	5460	*174*	6391	*1466*	***9***	***9***	**accepted**
Dispersivity	1.37	*0.04*	1.2	*0.2*	***24***	***24***	**accepted**
MW Range: High	14901	*179*	15560	*1084*	***12***	***9***	**accepted**
Low	2450	*144*	2305	*319*	***27***	***24***	**accepted**

Table D.9: Statistical Comparisons of MWD Characterization Data for original Ultrafiltration fraction F4 and Fraction F4 of 84.8 mg O_3/L ozonated ASB Effluent B (Data originally shown in tables 14 & 21)

	Eff F4		Ozonated F4		rank-sum	rank-sum	Ho
	mean	std dev	mean	std dev	calc.	@ 95%	
Distribution Area	1335	*27*	939	*38*	***21***	***24***	**rejected**
Weighted Average Molecular Weight	11551	*131*	10034	*140*	***21***	***24***	**rejected**
Number Average Molecular Weight	12178	*940*	12295	*1146*	***15***	***9***	**accepted**
Dispersivity	1.0	*0.1*	0.8	*0.1*	***23***	***24***	**rejected**
MW Range: High	32082	*3225*	34178	*8864*	***15***	***9***	**accepted**
Low	2866	*164*	2828	*152*	***29***	***24***	**accepted**

APPENDIX E

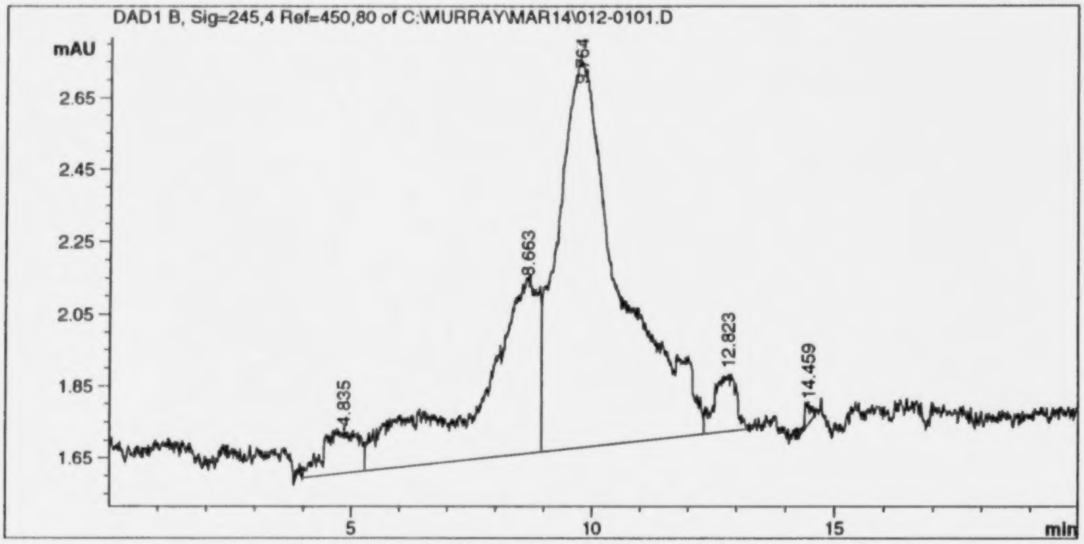
Standard Chromatograms

HPLC
CHROMATOGRAPHY REPORT

Injected on:	3/15/94 2:57:29 AM
Sample name:	Pullulan P-5
Sample info:	2.16% w/v in 25mM Na2CO3/NaHCO3
Vial#:	12
Injection#:	1

COLUMN:	Description:	Waters Ultrahydrogel 250
	Size:	7.8 mm ID x 30 cm

Mobile Phase:	25mM Carbonate Buffer (pH=10.2)
Flow:	0.8 mls/min
Pressure:	670 - 680 psi
Amount Injected:	20ul



Integration Parameters:	Area Reject	Peak Width	Threshold	Shoulders
	0.000	0.400	-5.5	OFF

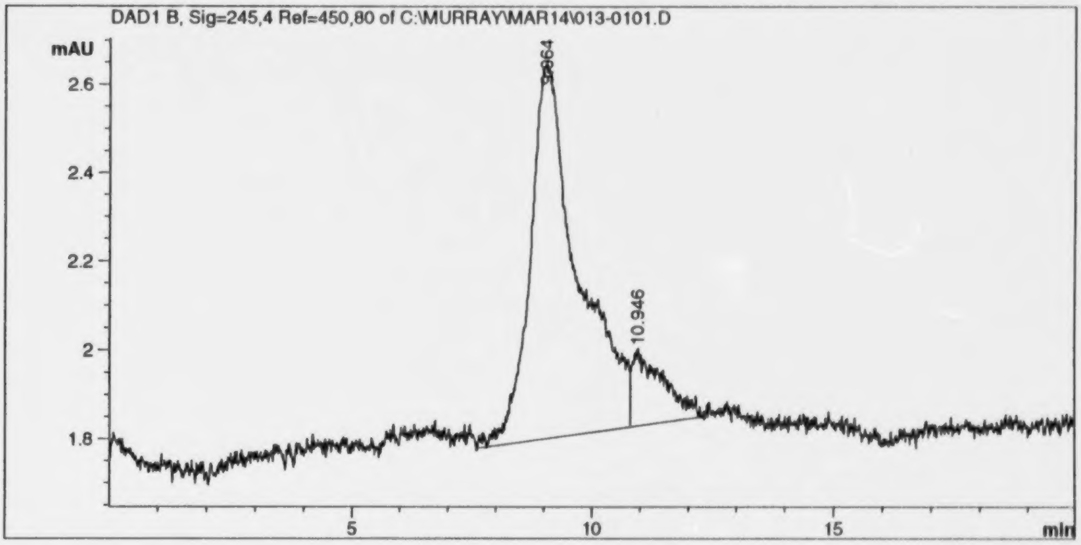
#	Ret.time [min]	Area [cnts*s]	Area%	Int.Type	Peak width [min]
1	4.835	6.2	4.1	PV	0.727
2	8.663	43.5	28.5	VV	1.140
3	9.764	97.5	63.8	VV	1.251
4	12.823	5.0	3.3	VV	0.445
5	14.459	0.6	0.4	BV	0.174
	Total Area [mAU*s]	152.7			

HPLC
CHROMATOGRAPHY REPORT

Injected on: 3/15/94 3:18:19 AM
Sample name: Pullulan P-10
Sample info: 1.93% w/v in 25mM Na2CO3/NaHCO3
Vial#: 13
Injection#: 1

COLUMN: Description: Waters Ultrahydrogel 250
Size: 7.8 mm ID x 30 cm

Mobile Phase: 25mM Carbonate Buffer (pH=10.2)
Flow: 0.8 mls/min
Pressure: 670 - 680 psi
Amount Injected: 20ul



Integration Parameters:	Area Reject	Peak Width	Threshold	Shoulders
	0.000	0.400	-5.5	OFF

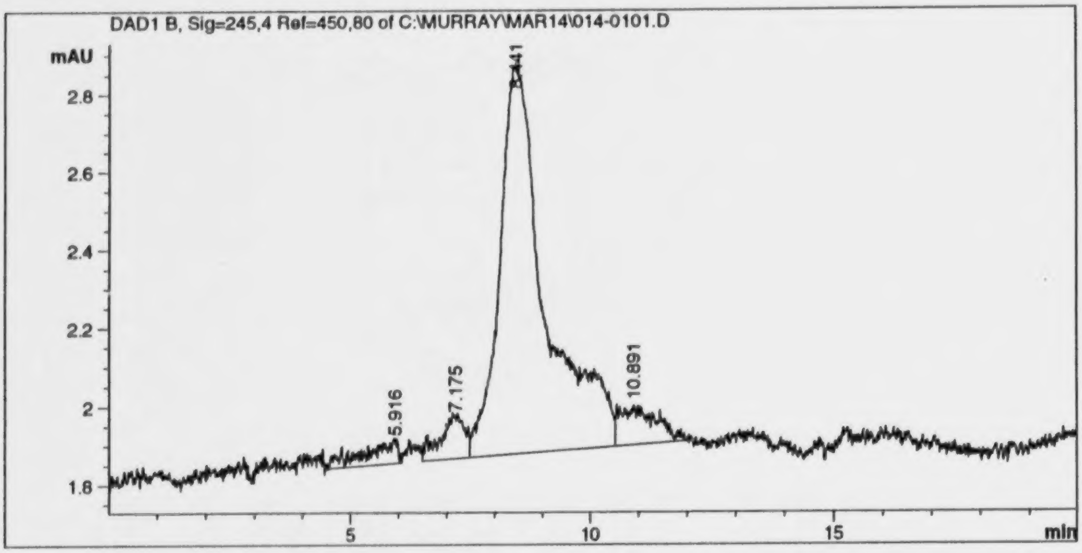
#	Ret.time [min]	Area [cnts*s]	Area%	Int.Type	Peak width [min]
1	9.064	58.7	88.4	PV	0.967
2	10.946	7.7	11.6	VV	0.598
	Total Area [mAU*s]	66.4			

HPLC
CHROMATOGRAPHY REPORT

Injected on: 3/15/94 3:39:10 AM
Sample name: Pullulan P-20
Sample info: 1.82% w/v in 25mM Na2CO3/NaHCO3
Vial#: 14
Injection#: 1

COLUMN: Description: Waters Ultrahydrogel 250
Size: 7.8 mm ID x 30 cm

Mobile Phase: 25mM Carbonate Buffer (pH=10.2)
Flow: 0.8 mls/min
Pressure: 670 - 680 psi
Amount Injected: 20ul



Integration Parameters:	Area Reject	Peak Width	Threshold	Shoulders
	0.000	0.400	-5.5	OFF

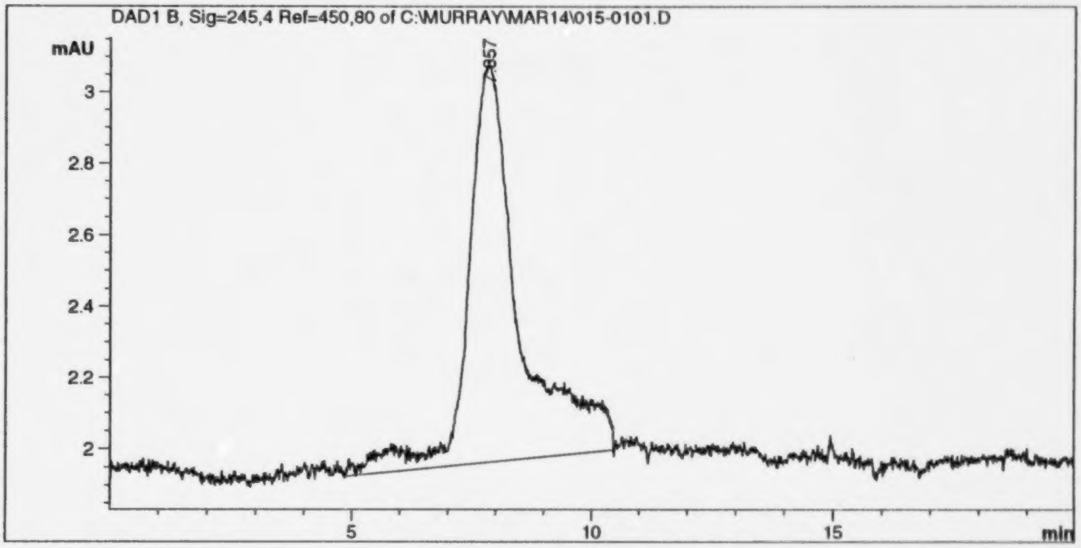
#	Ret.time [min]	Area [cnts*s]	Area%	Int.Type	Peak width [min]
1	5.916	2.9	3.7	VV	0.714
2	7.175	4.0	5.2	VV	0.516
3	8.441	65.6	84.4	VV	0.901
4	10.891	5.2	6.7	VB	0.862
	Total Area [mAU*s]	77.7			

HPLC
CHROMATOGRAPHY REPORT

Injected on: 3/15/94 4:00:00 AM
Sample name: Pullulan P-50
Sample info: 1.92% w/v in 25mM Na2CO3/NaHCO3
Vial#: 15
Injection#: 1

COLUMN: Description: Waters Ultrahydrogel 250
Size: 7.8 mm ID x 30 cm

Mobile Phase: 25mM Carbonate Buffer (pH=10.2)
Flow: 0.8 mls/min
Pressure: 670 - 680 psi
Amount Injected: 20ul



Integration Parameters:	Area Reject	Peak Width	Threshold	Shoulders
	0.000	0.400	-5.2	OFF

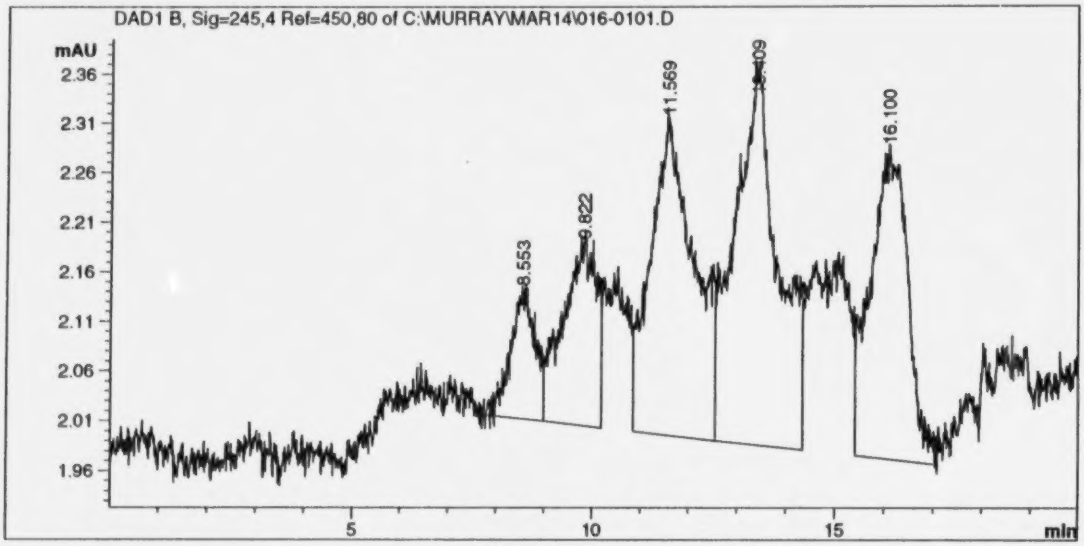
#	Ret.time [min]	Area [cnts*s]	Area%	Int.Type	Peak width [min]
1	7.857	83.5	100.0	VV	1.088
	Total Area [mAU*s]	83.5			

HPLC
CHROMATOGRAPHY REPORT

Injected on: 3/15/94 4:20:51 AM
Sample name: PEG Mixture
Sample info: 194;600;4100;12600 - 0.81;0.51;0.74;0.50% in CO3 buff.
Vial#: 16
Injection#: 1

COLUMN: Description: Waters Ultrahydrogel 250
Size: 7.8 mm ID x 30 cm

Mobile Phase: 25mM Carbonate Buffer (pH=10.2)
Flow: 0.8 mls/min
Pressure: 670 - 680 psi
Amount Injected: 20ul



Integration Parameters:	Area Reject	Peak Width	Threshold	Shoulders
	0.000	0.400	-5.2	OFF

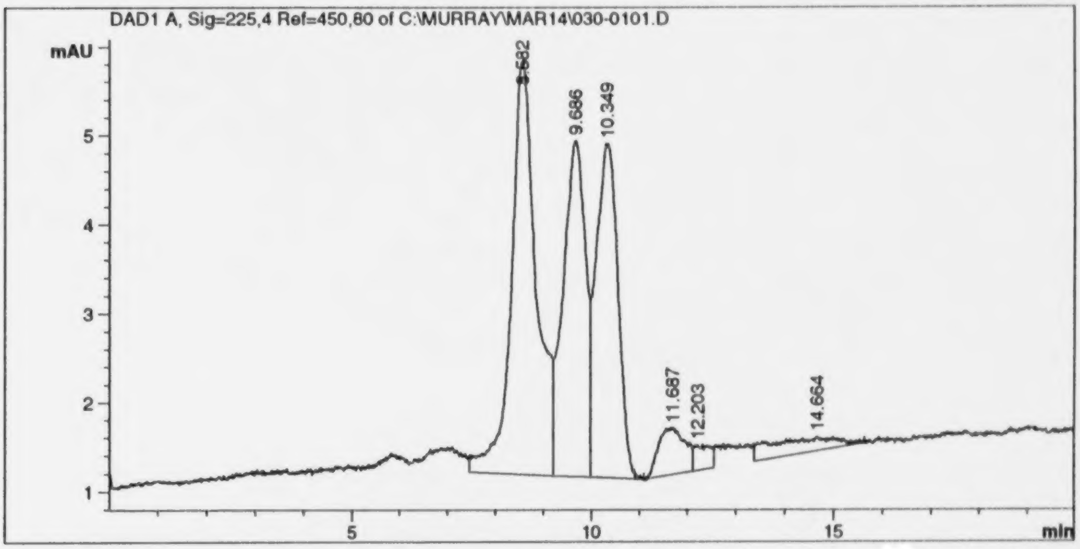
#	Ret.time [min]	Area [cnts*s]	Area%	Int.Type	Peak width [min]
1	8.553	4.6	6.3	BV	0.485
2	9.822	9.1	12.2	VV	0.634
3	11.569	20.1	27.1	VV	0.800
4	13.409	23.9	32.2	VV	0.804
5	16.100	16.5	22.2	VV	0.710
	Total Area [mAU*s]	74.2			

HPLC
CHROMATOGRAPHY REPORT

Injected on:	3/16/94 1:13:46 PM
Sample name:	Peptide Stds
Sample info:	dil 1:1:6 50mM : 25mM Na2CO3/NaHCO3
Vial#:	30
Injection#:	1

COLUMN:	Description:	Waters Ultrahydrogel 250
	Size:	7.8 mm ID x 30 cm

Mobile Phase:	25mM Carbonate Buffer (pH=10.2)
Flow:	0.8 mls/min
Pressure:	670 - 680 psi
Amount Injected:	20ul



Integration Parameters:	Area Reject	Peak Width	Threshold	Shoulders
	0.000	0.300	-4	OFF

#	Ret.time [min]	Area [cnts*s]	Area%	Int.Type	Peak width [min]
1	8.582	170.4	37.4	VV	0.511
2	9.686	122.6	26.9	VV	0.443
3	10.349	119.4	26.2	VV	0.439
4	11.687	20.3	4.5	PV	0.496
5	12.203	6.8	1.5	VV	0.393
6	14.664	16.3	3.6	VV	1.419

Total Area [mAU*s] 455.9